NTP Research Concept: Diethyl Phthalate (DEP)

Project Leader
Paul Foster, Ph.D.
DIR/ETP/TOB

Nomination Rationale and Background
Diethyl phthalate (DEP) is used primarily as a solvent in a number of applications, including personal care products and fragrances. It was nominated to the NTP for testing by NIEHS (http://ntp.niehs.nih.gov/go/29287). The CDC has been monitoring various members of the phthalate class of chemicals in the general US population for a number of years and DEP appears to have the highest concentration of any phthalate found in human urine (see for example (Blount *et al.* 2000; Calafat and McKee 2006; Silva *et al.* 2004a) and human amniotic fluid samples (Silva *et al.* 2004b).

Much of the recent biological concern with specific members of this class (e.g. Dibutyl and di(2-ethylhexyl) phthalates) has been related to their ability to produce a range of effects on reproductive development in rats due to their ability to interfere primarily with androgen action (Foster 2005, 2006) and that this phthalate syndrome of response (Gray and Foster 2003) has some parallels with the testicular dysgenesis syndrome reported for humans (Mahood *et al.* 2006; Skakkebaek *et al.* 2001; Virtanen *et al.* 2005). Specific phthalate esters produce these effects primarily via a lowering of fetal testicular testosterone levels and not through a direct interaction with the androgen receptor.

The NTP has previously conducted a reproductive assessment by continuous breeding study in mice that was without significant adverse effects (reported in (Lamb *et al.* 1987). However a close examination of the data from F_1 animals at the highest dose level (see Table 1) did reveal some statistically significant changes in reproductive parameters not noted in the parental generation. These changes occurred in the presence of body weight reductions, but at a very high dose level (2.5% in the diet). However, the mouse is recognized as being significantly more resistant to the antiandrogenic and other reproductive effects of phthalate esters than are rats. Indeed no studies indicating the pattern of reproductive tract malformations, characterized by multiple investigators in the rat, have been reported in the mouse.

Other studies have been conducted in the rat to look for antiandrogenic changes after perinatal exposure (usually single dose level, limited time window studies), either on malformation induction (Gray *et al.* 2000) or gene changes (Liu *et al.* 2005), without any significant effects being reported. DEP was also without response to testicular effects in the short-term rat pubertal model (Foster *et al.* 1980). A more recent dietary multigeneration study in the rat has been conducted (Fujii *et al.* 2005) and did not report any treatment related reproductive effects, but this did not include some of the endocrine end points known to be sensitive to specific phthalates (for example, retained nipples in males). Anogenital distance at birth, a good indicator of fetal androgen status, was measured and was not significantly altered by DEP treatment. Others have noted, however, that the power to detect post-natal developmental effects (e.g. epididymal malformations) in standard multigeneration studies is limited due to the selection process, with only one male and female pup being selected from each litter for examination in adult life (Gray

et al. 2004) compared to the normal developmental toxicity approach, where every fetus from each litter is examined.

There have been a number of small, human studies that have measured DEP exposure levels and have attempted to relate this to other endocrine-mediated developmental changes. Swan et al (Swan *et al.* 2005) found a positive correlation of reduced anogenital index (anogenital distance/bodyweight) in infant boys with increasing DEP metabolite urinary levels in mothers, which was confirmed in a follow-up report (Marsee *et al.* 2006). Another study of DEP metabolite in mothers' breast milk (Main *et al.* 2006) showed a positive correlation with the LH:free testosterone ratio in their 6 month old male infants. While the changes reported for both of these end points would be consistent with the effects of other phthalates on androgen signaling in rodents, these observations need to be confirmed in larger investigations. It should be noted that these studies did not produce any adverse effects on reproductive tract development in boys. Moreover, neither study showed any correlation with levels of DEHP metabolites (which were measured), a potent inducer of reproductive tract malformations when administered to rats during gestation. Indeed, it is likely that the correlations reported were more related to phthalate exposure, rather than any specific phthalate ester, since the strongest correlations seemed to follow the relative abundance of the specific phthalate metabolites.

Hypothesis

The null hypothesis is that DEP does not affect reproductive development in rats and is unlikely to present a risk to humans.

Proposed Research Project

Conduct a robust multigenerational study in the rat incorporating end points known to be sensitive to antiandrogenic phthalates. The study should be over a wide dose range and utilize an increased number of pups selected to be retained to adulthood for examination. A study with this type of design, conducted by the NTP on DEHP, has indicated a significantly increased ability to detect reproductive tract effects in the F_1 and F_2 generations at lower dose levels compared to the conventional multigeneration design (NTP, 2001).

Undertake toxicokinetic studies on dose delivery to the fetus (including amniotic fluid) and the pup for comparison to DEP human exposure studies.

Significance and Expected Outcome

These studies would fill the critical data gaps for DEP for which public health concern has been raised due to its higher human exposure compared to other members of this class and some small, human studies linking maternal exposure to DEP with alterations in androgen levels/ status in the offspring. The issue of cumulative risk for phthalate esters is gaining increased significance with regulatory agencies and it is crucial to be able to estimate whether DEP exposure contributes to any additional risks for individuals that have multiple phthalate metabolites in their tissues and fluids, as noted frequently in human monitoring studies.

References

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Table 1. Diethyl Phthalate RACB in the mouse: Evaluation of F_1 Offspring

Parameter	Control	DEP (2.5% = 4.2 g/kg/d)
Mating Index (%)	100 (20/20)	100 (20/20)
Fertility index (%)	95 (19/20)	95 (19/20)
Live pups per litter	11.53 ± 0.54	9.95 ± 0.67 *
Live pup weight (female) g	1.50 ± 0.03	1.42 ± 0.03*
Male Body weight at necropsy (g)	34.16 ± 0.81	30.20 ± 0.61 *
Female Body weight at necropsy (g)	30.57 ± 0.61	28.21 ± 0.51 *
Male Liver wt (adjusted) g	1.71 ± 0.04	2.01 ± 0.04 *
Female Liver wt (adjusted) g	1.74 ± 0.04	2.22 ± 0.04 *
Sperm Concentration (10^6/ mg cauda)	1021.17 ± 47.95	718.07 ± 36.23 *

* $p<0.05$