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Subject:	mechanism of action for phthalates
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Attachments:	pgs A4 to A5 from CPSC Report Appendix-A-Developmental-FINAL.PDF

Dear Mike,

As you know, one of the subjects discussed at the IRIS meeting last week was the mode of action for male reproductive effects in rats associated with phthalate treatment. Dr. Foreman showed a table (attached) that was abstracted from the CHAP report in which differences in gene expression profiles for DINP and DEHP were summarized. Dr. Gray took issue with this table, stating that he had data (apparently unpublished), that contradicted the information provided. After the meeting we took another look at the CHAP report. As best we can tell, the DINP data that are summarized in the table are not referenced in the text. We were hoping that you could help us find the source for the information and if it has not been published whether you could arrange to have it made available for review during the IRIS process.

As mode of action information for DINP seems very relevant to both the CHAP and the IRIS processes, we would be grateful for any help you could provide in helping us identify the source of the information.

Regards

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1.4 Mechanism of Action

Initial mechanistic studies centered on phthalates acting as environmental estrogens or antiandrogens; however, data from various estrogenic and antiandrogenic screening assays clearly showed that while the parent phthalate could bind to steroid receptors, the developmentally toxic monoesters exhibited little or no affinity for the estrogen or androgen receptors (David, 2006). Another potential mechanism of phthalate developmental toxicity is through peroxisome proliferator-activated receptor alpha (PPARα). Support for this hypothesis comes from data showing that circulating testosterone levels in PPARα-null mice were increased following treatment with di(2-ethylhexyl) phthalate (DEHP) compared with a decrease in wild-type mice, suggesting that PPARα plays a role in postnatal testicular toxicity. PPARα activation may play some role in the developmental toxicity of nonreproductive organs (Lampen *et al.*, 2003); however, data linking PPARα activation to the developmental toxicity of reproductive organs are lacking.

Because other studies had shown that normal male rat sexual differentiation is dependent upon three hormones produced by the fetal testis, *i.e.*, an anti-Mullerian hormone produced by the Sertoli cells, testosterone produced by the fetal Leydig cells, and insulin-like hormone 3 (insl3), several laboratories conducted studies to determine whether the administration of specific phthalates to pregnant dams during fetal sexual differentiation that caused demasculinization of the male rat offspring would also affect testicular testosterone production and insl3 expression. Studies by Wilson *et al.* (2004), Howdeshell *et al.* (2007), and Borch *et al.* (2006b) reported significant decreases in testosterone production and insl3 expression after DEHP, DBP, and butylbenzyl phthalate (BBP), and by DEHP + DBP (each at one-half of its effective dose). The study by Wilson *et al.* (2004) also showed that exposure to DEHP (and similarly to DBP and BBP) altered Leydig cell maturation, resulting in reduced production of testosterone and insl3, from which they further proposed that the reduced testosterone levels result in malformations such as hypospadias, whereas reduced insl3 mRNA levels lead to lower levels of this peptide hormone and abnormalities of the gubernacular ligament (agenesis or elongated and filamentous) or freely moving testes (no cranial suspensory or gubernacular ligaments). Together, these studies identify a plausible link between inhibition of steroidogenesis in the fetal rat testes and alterations in male reproductive development. In addition, other phthalates that do not alter testicular testosterone synthesis (diethyl phthalate [DEP]; Gazouli *et al.*, 2002) and gene expression for steroidogenesis (DEP and dimethyl pthalate [DMP]; Liu *et al.*, 2005) also do not produce the phthalate syndrome malformations produced by phthalates that do alter testicular testosterone synthesis and gene expression for steroidogenesis (Gray *et al.*, 2000; Liu *et al.*, 2005).

Complementary studies have also shown that exposure to DBP *in utero* leads to a coordinated decrease in expression of genes involved in cholesterol transport (peripheral benzodiazepine receptor [PBR], steroidogenic acute regulatory protein [StAR], scavenger receptor class B1 [SR-B1]) and steroidogenesis (Cytochrome P450 side chain cleavage [P450scc], cytochrome P450c17 [P450c17], 3β-hydroxysteroid dehydrogenase [3β-HSD]), leading to a reduction in testosterone production in the fetal testis (Shultz *et al.*, 2001; Barlow and Foster, 2003; Lehmann *et al.*, 2004). Interestingly, Lehmann *et al.*, (2004) further showed that DBP induced significant reductions in SR-B1, 3β-HSD, and c-Kit (a stem cell factor produced by Sertoli cells that is essential for normal gonocyte proliferation and survival) mRNA levels at doses (0.1 or 1.0

mg/kg-d) that approach maximal human exposure levels. The biological significance of these data are not known, given that no statistically significant observable adverse effects on male reproductive tract development have been identified at DBP dose <100 mg/kg-d and given that fetal testicular testosterone is reduced only at dose levels equal to or greater than 50 mg/kg-d.

Thus, current evidence suggests that once the phthalate monoester crosses the placenta and reaches the fetus, it alters gene expression for cholesterol transport and steroidogenesis in Leydig cells. This, in turn, leads to decreased cholesterol transport and decreased testosterone synthesis. As a consequence, androgen-dependent tissue differentiation is adversely affected, culminating in hypospadias and other features of the phthalate syndrome. In addition, phthalates (DEHP and DBP) also alter the expression of insl3, leading to decreased expression. Decreased levels of insl3 result in malformations of the gubernacular ligament, which is necessary for testicular descent into the scrotal sac.

Summary of Mechanism of Action Studies									
Chemical	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
DBP	↓	↓		↓		↓	↓	↓	
BBP	↓	↓							
DEHP	↓	↓	↓	↓	↓	↓	↓	↓	↓
DEHP+DBP	↓	↓	↓	↓					
DNOP									
DINP	↓	↑	↓	↓	↑			↑	
DIDP									
DMP									
DEP									
DIBP	↓	↓		↓		↓		↓	↓
DPENP	↓	↓	↓	↓					
ATBC									
DEHA									
DINX									
DEHT									
TOTM									
TPIB									

1 = Testosterone
2 = insl3 (Insulin-like factor 3)
3 = CYP11A (Rate-limiting enzyme responsible for the conversion of cholesterol to pregnenolone)
4 = StAR = Steroidogenic Acute Regulated Protein, involved in mitochondrial cholesterol uptake
5 = LH = Lutenizing Hormone
6 = SR-B1 = Scavenger Receptor B-1, responsible for cholesterol uptake by Leydig cells
7 = PBR = Peripheral Benzodiazepene Receptor, involved in mitochondrial cholesterol uptake
8 = CYP450scc = Cytochrome P450 side chain cleavage enzyme, steroid converting enzyme
9 = SF-1 = Nuclear Receptor Steroidogenic Factor-1, regulates expression of genes involved in steroidogenesis