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Re: Toxicology Study Nomination of Diethyl Phthalate

Dear Dr. Masten:

The Phthalate Esters Panel of the American Chemistry Council[1] submits this letter to the National Toxicology Program (NTP) in response to its recent request for public comment on its toxicological study nomination of certain chemicals, including diethyl phthalate (DEP). 72 Fed. Reg. 14816 (Mar. 29, 2007). NTP has nominated DEP for additional multigeneration oral reproductive and developmental studies and toxicokinetic studies by the oral and dermal route, citing as its rationale widespread consumer exposure to DEP from cosmetics and insufficient reproductive toxicity data. As explained in this letter, the Panel believes that neither of these rationales justifies the time and expense or the sacrifice of animals necessary to conduct the additional proposed studies. The Panel strongly supports the regulation of chemicals based on sound science, and to promote such regulation has over the past three decades sponsored numerous toxicological studies of DEP and other phthalates. In the case of DEP, the Panel believes the existing toxicological database is sufficient to assess its reproductive toxicity and that the additional toxicological studies proposed by NTP are unnecessary and would represent a significant investment of resources that would be better allocated elsewhere.

The Toxicological Database for DEP is Sufficient to Assess its Potential Reproductive Hazard and Indicates that DEP is Not a Developmental or Reproductive Toxicant

As demonstrated in NTP's DEP Chemical Information Profile,[2] DEP has an extensive toxicological database that contains several reproductive and developmental toxicity studies, including two separate multigeneration reproductive toxicity studies. Together, these studies are sufficient to assess the developmental and reproductive toxicity of DEP, and they indicate that DEP is not a developmental or reproductive toxicant and that no additional studies are needed.

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1 The Panel members are BASF Corporation, Eastman Chemical Company, ExxonMobil Chemical Company, Ferro Corporation and Teknor Apex Company.

2 Integrated Laboratory Systems, Inc., Chemical Information Profile for Diethyl Phthalate: Supporting Information for Toxicological Evaluation by the National Toxicology Program. (November 2006). Available at: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ ExSumPdf/Diethyl_phthalate.pdf

A recent two-generation study found no reproductive or developmental effects from DEP exposure. Fujii et al. (2005) fed male and female rats DEP at concentrations of 0, 600, 3000 and 15000 ppm for 10 weeks prior to mating, and throughout mating, gestation and weening for two generations. There were no significant changes in reproductive function for the F_0 or F_1 animals at any dose. F_0 males had a slight, but not significant, decrease in circulating testosterone at the two highest doses, 3000 and 15000 ppm. The authors also reported a slight, but not significant, delay in bodyweight gains in F_1 and F_2 pups, and vaginal opening in F_1 females. In summary, this two-generation study, which NTP characterized as "well-conducted," found few effects of any kind, and no significant adverse effects on reproductive function at doses up to 15000 ppm. If there are any "limitations" to the design of this study, as suggested by NTP, they are effectively rendered insignificant by the corroborating results of another multigeneration study and several other reproductive or developmental studies.

In a second multigeneration study, NTP evaluated DEP for reproductive function and fertility effects in a mouse continuous breeding study (NTP, 1984). In that study, CD-1 mice were fed DEP in their diet at concentrations up to 2.5%. The study design allowed parental exposure (F_0 generation) to the test material prior to mating, during mating, gestation and during lactation of the F_2 generation pups. The F2 generation mice, therefore, were exposed to the test material from conception via their parents, during gestation and lactation via the F_0 mother, and directly post-nataly via dosed-feed during the later portion of lactation and, eventually, directly through feeding during their period of sexual maturation and breeding. There were no adverse effects of DEP treatment on reproductive parameters or fertility of the F_0 parental generation. Reproductive parameters included successful mating, number of litters produced, proportion of live pups per litter, sex ration of pups, pup weight. The offspring of the F_0 parents did show signs of systemic toxicity. At weaning, male and female high-dose F_1 mice had reduced body weight and increased (relative) liver weight compared to control animals and males showed an increased prostate weight while females showed decreased pituitary weights. When these animals were mated to produce the F_2 generation reproductive effects were observed in the high-dose group: a reduced number of pups per litter was reported.

The dose levels employed in this study were remarkably high by any standards and especially as compared to high-dose limits described in current regulatory protocols. The high-dose group in the NTP continuous breeding study described above received a diet containing 2.5% phthalate. This concentration equates to approximately 5125 mg/kg/day of DEP in mature mice, a dose level 5-fold above that which would be considered a limit dose in current studies of reproductive function. OPPTS guideline for reproductive and fertility effects assessment studies of rodents (OPPTS Guideline 83-4, OPPTS Number 870-3800) indicates that "the highest dose tested should not exceed 1,000 mg/kg/day or 20,000 ppm in the diet, unless potential human exposure data indicate the need for higher doses." Clearly the high dose used in the NTP continuous breeding study produced systemic and reproductive toxicity, and equally clearly lower doses (0.25% and 1.25% dietary did not produce reproductive toxicity or impair fertility. Accordingly, the NTP multigeneration study indicates that DEP is not a developmental or reproductive toxicant at realistic possible exposure levels.

NTP also conducted a developmental toxicity study using pregnant CD rats administered oral doses of DEP to timed-pregnant female rats at concentrations of 0, 0.25, 2.5 and 5% DEP in the feed (0, 198, 1909 and 3214 mg/kg/day) on gestational days (GD) 6-15. (NTP, 1988; Field et al., 1993). Maternal toxicity was evident as a decrease in body weight in the 5% DEP group

through day 15. The 2.5% DEP group had a transient decrease in maternal body weight at the beginning of dosing while weight gain was observed in the 0.25% DEP group. There also was a transient decrease in food and water consumption in the 2.5 and 5% DEP groups at the start of dosing. However, there was no evidence of changes in embryo/fetal growth, viability or the incidence of malformations at any dose. There was an increased incidence in a variation (extra rib) at 5% DEP, but only at a dose that also caused maternal effects and therefore is not considered biologically significant. This developmental study thoroughly evaluated the effects on organogenesis of major systems – corroborating the negative results of the multigeneration studies – but it did not directly address concerns about the developing male reproductive tract. Those concerns were addressed in more recent studies.

Gray et al. (2000) administered several phthalates, including di(2-ethylhexyl) phthalate (DEHP), butylbenzyl phthalate (BBP), diisononyl phthalate (DINP) and DEP to pregnant rats and examined sexual differentiation of the pups. Groups of timed-pregnant female SD rats were administered individual phthalates at doses of 0 or 750 mg/kg/day in corn oil from GD 14 to postnatal day 3 (PND 3). No overt indicators of maternal toxicity were observed. Although effects were seen in pups from dams exposed to DEHP, BBP and DINP (changes in anogenital distance and decreased testes weights in pups from dams treated with DEHP and BBP, and female-like areolas/nipples and reproductive malformations in pups from dams treated with DEHP, BBP and DINP), treatment with DEP again resulted in no developmental or reproductive effects.

The lack of reproductive or developmental toxicity of DEP has been confirmed by gene expression studies. Liu et al. (2005) performed gene expression profiling on two groups of phthalates: those previously associated with reproductive toxicity in rodents (DEHP, DBP, dipentylphthalate, and BBP) and those not associated with reproductive toxicity (dimethyl phthalate (DMP), DEP and diethylhexyl terephthalate (DOTP)). Groups of pregnant SD rats were treated with either corn oil vehicle or the appropriate phthalate (500 mg/kg/day) on GD 12-19. At sacrifice, testes of male pups were collected and gene profiling was performed. The results showed that the phthalates commonly associated with reproductive toxicity produced an identical pattern of gene expression change in the testes. Conversely, the phthalates not commonly associated with reproductive toxicity, specifically DEP, produced a pattern of gene expression different from those associated with reproductive toxicity. As predicted by the authors, the changes in gene expression for the phthalates associated with reproductive toxicity included genes involved in cholesterol transport and steroidogenesis, among others. Thus, rodent gene expression data support the findings of experimental animal studies that DEP is not a reproductive or developmental toxicant.

In vitro studies also provide evidence that DEP does not affect testosterone production. In particular, Gazouli et al. (2002) used cultured Leydig cells to demonstrate that DEP did not alter parameters of cellular function or testosterone secretion. In this model, peroxisome proliferators inhibit lipid metabolic pathways and decrease the production of testosterone in cultured Leydig cells. While the positive controls DEHP and benzofibrate significantly inhibited testosterone secretion, DEP had no effect on testosterone secretion. Similarly, Lampen et al. (2003) demonstrated that DEP was not able to activate peroxisome proliferator-activated receptors α, γ or δ in cultured F9 cells. This model uses the differentiation of a mouse blastocyte-like cell to screen for teratogenic chemicals. DEP did not induce differentiation in F9

cells, while the positive control, valproic acid, and other phthalates associated with reproductive toxicity in rodents, did induce differentiation.

The above studies clearly demonstrate that DEP is not associated with reproductive toxicity either in animal models or using in vitro assays. DEP did not cause reproductive toxicity in two separate multi-generation studies (at any level approaching a realistic dose) or in developmental reproductive studies, and treatment of cells with DEP did not result in the same pattern of gene expression that is unique to phthalates known to produce reproductive toxicity, nor did it inhibit testosterone production in cell-based assays. In summary, the toxicological database for DEP is robust, and shows that DEP is not a reproductive toxicant.

Statistical Correlation Studies Provide No Data Suggesting Additional Studies are Required

In addition to the above experimental studies, several reports have been published that present anecdotal statistical relationships between exposure to DEP (or phthalates in general) and various reproductive endpoints. Hauser et al., (2007) reported a significant statistical association between levels of a DEP metabolite and DNA damage in human sperm. The subjects used were 379 men from an infertility clinic with no control group of men with normal reproductive function. In another study, Hauser et al. (2006) did not demonstrate an association between semen quality and levels of DEP metabolites in the urine. The subjects were 463 males from subfertile couples and a group of control men. Swan et al., (2005) reported a significant inverse relationship between DEP metabolite concentration in urine and ano-genital index in 134 young boys (age 2-36 months). Duty et al., (2003) published a study with similar methodology and results to the Hauser (2007) study, and showed a statistically significant association between urinary DEP metabolites and sperm DNA damage. There was no correlation between metabolites of DEHP, DMP, DBP and BBP and sperm DNA damage.

In general, the above statistical studies provide results that are anecdotal in nature. They show a statistical association between a common chemical, or class of chemicals used in personal care products, and a selected reproductive parameter. However, there is no causal relationship established, and there is no evaluation of other common, non-phthalate environmental chemicals. The latter evaluation would be necessary to establish that the increases in phthalate levels were not simply a biomarker of exposure to environmental chemicals in general, as opposed to a specific toxicant. For example, studies showing DEP to be non-mutagenic support the theory that DEP is a biomarker rather than a proximate toxicant for DNA damage. In the Swan article, the primary discrepancy is that there is no way to determine maternal phthalate levels during critical windows of development for a given teratogenic event since the levels of phthalate in the body would be expected to increase or decrease on a daily basis relative to product uses. Moreover, EPA has found that Swan and other epidemiological studies purporting to show a correlation between phthalate exposure and reproductive effects are unsuitable for use in the risk assessment process because they cannot demonstrate causation.[3] As such, these studies provide no indication that additional DEP toxicological studies are needed.

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[3] See, EPA Draft Toxicological Review of Dibutyl Phthalate (Di-n-Butyl Phthalate): In Support of the Summary Information in the Integrated Risk Information System(IRIS), available at: http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=457421.

Measured DEP Exposure Levels Do Not Indicate that Additional Studies Are Required

NTP's second justification for proposing additional DEP studies is that exposures to DEP from cosmetics are widespread and higher than other phthalates. Centers for Disease Control and Prevention (CDC) biomonitoring data show that exposures to DEP, whether widespread or not, are well below EPA's reference dose (RfD) for DEP of 800 µg/kg/day, which is based on non-reproductive effects in rats. Calculations based on biomonitoring results from 2782 individuals in the CDC's Third National Report on Human Exposure to Environmental Chemicals[4] show that both mean (5.5 µg/kg/day) and 95th percentile (61.7 µg/kg/day) exposures to DEP are well below EPA's RfD (800 µg/kg/day), and that 95th percentile exposures to women (47.4 µg/kg/day), who presumably use more cosmetics than men, are actually below exposures to men (69.0 µg/kg/day).[5] These low levels of exposure to DEP in the general population, particularly in relation to EPA's RfD, do not suggest any particular need for additional toxicological studies.

No Toxicokinetic Studies are Required for DEP

NTP also proposes to conduct oral and dermal toxicokinetic studies for DEP. As acknowledged in the Chemical Information Profile, the robust database for DEP includes several dermal absorption studies (Scott et al., 1987; Elsisi et al., 1989; Mint et al., 1994; Tokunaga et al., 2001). However, the Chemical Information Profile does not reference a study that has already investigated DEP toxicokinetics (Sapota et al., 2000). Moreover, as explained above, the extensive scientific database on DEP indicates that DEP is not a reproductive or developmental toxicant. Therefore, the Panel believes that the resources necessary to conduct such toxicokinetic studies would be better applied to other substances.

Conclusion

The Panel has for decades supported the development of complete toxicological databases for all phthalates. The Panel believes that the toxicological database for DEP is sufficient to assess its potential reproductive hazard. This database includes two separate multi-generation studies, developmental reproductive studies, and in vitro assays investigating both changes in gene expression and testosterone production in response to DEP. All these studies indicate that DEP is not a developmental or reproductive toxicant. Consequently, the Panel believes that additional reproductive and developmental studies and toxicokinetic studies are not warranted, and that the resources required to perform such studies would be more constructively applied elsewhere.

Sincerely,

[Redacted]

Marian Stanley,
Manager, Phthalate Esters Panel

Attachment

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4 Available at: http://www.cdc.gov/exposurereport/report.htm.

5 Calculated using the methodology described in Calafat and McKee, (2006). Available at: http://ehp.niehs.nih.gov/members/2006/9059/9059.html.

ATTACHMENT

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