

This document was developed by NIEHS/NTP staff to facilitate internal and external review of a proposed research program prior to designing and conducting toxicology studies. The purpose of the research concept document is to outline the general elements of a research program that would address the specific public health concerns that prompted the nomination of the substance or issue for study. It may also encompass substance-specific studies that address larger public health issues or topics in toxicology. Additional information about the nomination, review, and selection of substances for study by the NTP is provided at *Nominations to the NTP Testing Program* (<http://ntp.niehs.nih.gov/go/nom>). A draft version of this research concept was reviewed by the NTP Board of Scientific Counselors at a public meeting on December 6, 2007 (<http://ntp.niehs.nih.gov/go/9741>) and subsequently revised.

## **NTP Research Concept: Di(2-ethylhexyl) Phthalate (DEHP) and Phthalate Mixtures**

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### **Nomination History**

Di(2-ethylhexyl) phthalate (DEHP) and other phthalates have been nominated on a number of occasions to the NTP for testing. In particular many aspects of the research proposed in this document would fall under the nominations that were previously approved by the BSC for the study of peroxisome proliferators (initiated in the 1990's), the nomination of DEHP by FDA in 2004 and the critical data needs highlighted in the NTP Center for the Evaluation of Risks to Human Reproduction monograph on DEHP issued in 2006.

### **Background**

DEHP is a ubiquitous environmental contaminant that has been shown to produce reproductive, developmental and cancer effects in rodents. The cancer risk assessments conducted by a number of different regulatory authorities have changed over time with the advent of detailed mechanistic information on the involvement of PPAR $\alpha$  (peroxisome proliferator activated receptor alpha) in the carcinogenic process. In 1992, based on hepatocarcinogenesis in rodents (predominantly from NTP studies) the EPA and then IARC classified DEHP in category 2. Much later, a paper (Doull *et al.* 1999) proposed that the liver tumors were due to PPAR $\alpha$  activation and that this mechanism was not relevant for humans and should not be used in human risk assessment. This mechanistic body of work resulted in the delisting of DEHP by IARC (IARC 2000) and the European Union (CSTEE 2004) as a potential carcinogen (i.e. category 3).

Since this time a further paper in the Sprague-Dawley rat has indicated that the liver is not the sole target for DEHP carcinogenicity in lifetime studies, with testicular as well as liver tumors also being observed (Voss *et al.* 2005). Pancreatic acinar adenomas have also been reported as treatment related findings in chronic studies in the F-344 rat (David *et al.* 2000). Moreover, a recent paper in which PPAR $\alpha$ -null mice were exposed to DEHP for 22 months (Ito *et al.* 2007), indicated that more liver tumors occurred in the null mouse than in the wild type animals. These data would imply that factors other than PPAR $\alpha$  are involved in DEHP hepatocarcinogenesis, as has been suggested by others (Melnick 2002; Melnick *et al.* 2003).

When rats are exposed *in utero* to DEHP, this agent produces a range of developmental effects including lowered fetal testosterone levels, anti-androgenic phenotypes and reproductive tract malformations (Gray *et al.* 2000) in an identical manner to that observed for di-*n*-butyl phthalate (DBP) (Foster 2005, 2006). DBP produces testicular dysgenesis in rats that results in Leydig cell

tumors of the testis in long term follow-up of exposed offspring after only a 10-day exposure *in utero* (Barlow *et al.* 2004).

PPAR $\alpha$  has been associated with developmental toxicity produced by other agents, including perfluorooctanoic acid (PFOA). The developmental toxicity of PFOA has been examined in PPAR $\alpha$ -null mice (Abbott *et al.* 2007) and while the postnatal manifestations of PFOA (early pup death) were not seen in the null mice, the *in utero* developmental effects of PFOA were observed (embryo-fetal death). These data would imply that PPAR $\alpha$  expression is developmentally regulated in the mouse. Thus, a critical issue for future risk assessments of DEHP will be the influence of exposure throughout different developmental ages (including the perinatal period) and determining the role, if any, of PPAR $\alpha$  in these responses, since the information on mechanistic relevance used in the current risk assessments by IARC and the EU appears flawed. The use of DEHP as a model compound may have applicability to other phthalate esters to which we know humans are exposed and potentially other PPAR $\alpha$  ligands.

The CDC has been monitoring exposure to various phthalates (including DEHP) in human urine as part of the National Health and Nutrition Examination Survey (NHANES) efforts and has noted a high frequency of exposures to multiple phthalates in the general population (see for example (Blount *et al.* 2000; Calafat and McKee 2006; Silva *et al.* 2004a). In a much smaller study, multiple phthalate metabolites have also been measured in human amniotic fluid samples (Silva *et al.* 2004b). Such samples potentially provide the best estimates of exposure for human fetuses that could be used in direct comparison to the levels found in the amniotic fluid of rodents at dose levels that can induce reproductive tract malformations (Calafat *et al.* 2006).

An important issue in any risk assessment for phthalate esters, is what is the contribution of mixed phthalate exposures to adverse outcomes? Recent papers have indicated that the *in utero* effects of mixtures of phthalates (Howdeshell *et al.* 2007) or antiandrogens (Metzdorff *et al.* 2007) show dose additivity in response.

### **Proposed Approach**

Hypotheses to evaluate are:

- That lifetime (perinatal + 2 year) exposure to DEHP would impact the dose response, incidence and/or severity for cancers of the liver and testis (and perhaps the pancreas) compared with adult only exposure.
- That PPAR $\alpha$  is developmentally regulated in the rat and unlikely to contribute to toxicity initiated *in utero* after exposure to DEHP.
- That exposures to mixtures of phthalates, based on their individual potencies, would result in dose addition for cancer outcomes

### **Specific Aims**

1. Undertake a “perinatal” cancer bioassay with DEHP in the Wistar Han rat to address any additional contribution of early life exposure to cancer outcome after exposure *in utero*, in early life and as an adult. This would allow a more complete assessment to be made of carcinogenic potential and should allow the evaluation of targets other than the liver. The Wistar is known to respond to the effects of DEHP *in utero* (Wilson *et al.* 2007). This

perinatal study should be compared to an “adult only” study in the same strain to address directly hypothesis 1. Selection of appropriate interim time points up to 2 years after birth may address both PPAR $\alpha$ -dependent and PPAR $\alpha$ -independent mechanisms that are considered relevant to the development of tumors in liver and in other target tissues. Consideration of the Sprague-Dawley strain (which has shown liver and testicular tumors in long term studies) should also be addressed.

2. Undertake an ontogeny study of PPAR $\alpha$  in the Wistar Han rat. Such a study would determine when the receptor is first expressed in target tissues to complement the PPAR $\alpha$  null mouse work conducted with PFOA. Since the antiandrogenic effects of DEHP (and other active phthalates) are not found in the mouse, the use of a PPAR $\alpha$  null mouse approach *in utero* would not yield the toxicity information required. While experiments in the PPAR $\alpha$  null mouse, or mice with “humanized PPAR $\alpha$ ” may still provide some useful information on the potential for DEHP to induce liver tumors, this approach will not address the pancreatic and testicular tumor issues (since these are not noted in mice). The role of other PPAR isoforms in toxicity is not clear, although some phthalates are ligands for these receptors. Studies on receptor expression in target tissues could therefore also include PPAR $\alpha$  and PPAR  $\delta/\gamma$ .
3. As a second tier of study, it is proposed to undertake perinatal phthalate mixture studies using the Toxic Equivalency Factor (TEF)-type approach. Such studies should be approached with care. In particular there are a number of specific issues that require consideration:
  - a. Route of exposure and associated kinetics. Choice of route of exposure would be very important (diet vs. gavage). To obtain more precision of external dose and to minimize dose intervals (there is a large variability in diet consumption during pregnancy and lactation that is not mirrored by bodyweight changes), gavage should be considered. To support these studies, TK data and estimates of internal dose are required in the Wistar rat during pregnancy and lactation by both gavage and dietary routes.
  - b. Short-term assays on a number of phthalates (e.g. dibutyl (DBP), diisobutyl (DiBP), butylbenzyl (BBP), diisononyl (DINP) and DEHP) would be required to develop potency estimates in the Wistar (Han) rat. For *in utero* exposures, the potency estimates would be via measurements of fetal testicular testosterone levels. For weanlings, some estimates of hepatic peroxisome proliferator activity would be required (e.g. CYP 4A1, Palmitoyl CoA Oxidase etc). It is anticipated that no more than 3 phthalates would be evaluated in any long-term mixture study.
  - c. Individual TK data on esters that were selected to go forward to longer-term studies would be required.

These data would guide the needs for individual perinatal bioassays and mixture work to support the DEHP study identified above. Since the question of cumulative risk for phthalates has been submitted recently to the NAS by EPA, this overall approach is seen as providing extra impetus to fill these data gaps.

## **Significance and Expected Outcome**

Such studies would:

- Provide a cancer hazard assessment for lifetime exposure to DEHP and address some of the critical questions posed with regard to the influence of early exposures on cancer outcome.
- Elucidate the developmental ontogeny of PPAR $\alpha$  in the rat and relationship to DEHP cancer (and other developmental toxicity) outcomes.
- Provide toxicity data on important environmental phthalates during lifetime exposures (perinatal + 2 years). In addition, to provide the critical data to undertake mixture studies using the TEF approach, to inform on potential cumulative and aggregate cancer risk. Recent data have indicated that because of similar modes of action *in utero*, phthalate esters do show dose addition when administered in combination and thus it would be appropriate to consider cumulative risk for the class since human subjects (including fetuses) are typically exposed to multiple phthalates.

## **References**

Abbott, B. D., Wolf, C. J., Schmid, J. E., Das, K. P., Zehr, R. D., Helfant, L., Nakayama, S., Lindstrom, A. B., Strynar, M. J., and Lau, C. S. (2007). Perfluorooctanoic Acid (PFOA)-induced Developmental Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated Receptor-alpha (PPAR{alpha}). *Toxicol Sci* **98**, 571-581.

Barlow, N. J., McIntyre, B. S., and Foster, P. M. D. (2004). Male Reproductive Tract Lesions at 6, 12, and 18 Months of Age Following in Utero Exposure to Di(n-butyl) Phthalate. *Toxicol Pathol* **32**, 79-90.

Blount, B. C., Silva, M. J., Caudill, S. P., Needham, L. L., Pirkle, J. L., Sampson, E. J., Lucier, G. W., Jackson, R. J., and Brock, J. W. (2000). Levels of seven urinary phthalate metabolites in a human reference population. *ENVIRONMENTAL HEALTH PERSPECTIVES* **108**, 979-982.

Calafat, A. M., Brock, J. W., Silva, M. J., Gray, L. E., Jr., Reidy, J. A., Barr, D. B., and Needham, L. L. (2006). Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate. *Toxicology* **217**, 22-30.

Calafat, A. M., and McKee, R. H. (2006). Integrating Biomonitoring Exposure Data into the Risk Assessment Process: Phthalates [Diethyl Phthalate and Di(2-ethylhexyl) Phthalate] as a Case Study. *Environ Health Perspect* **114**, 1783-9.

CSTEE (2004). Opinion on the results of a second risk assessment of: bis(2-ethylhexyl)phthalate (DEHP). Scientific Committee on Toxicity, Ecotoxicity and the Environment: Adopted by the CSTEE during the 41st plenary meeting of 8 January 2004. *European Commission*.

David, R. M., Moore, M. R., Finney, D. C., and Guest, D. (2000). Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *TOXICOLOGICAL SCIENCES* **55**, 433-443.

Doull, J., Cattley, R., Elcombe, C., Lake, B. G., Swenberg, J., Wilkinson, C., Williams, G., and van Gemert, M. (1999). A Cancer Risk Assessment of Di(2-ethylhexyl)phthalate: Application of the New U.S. EPA Risk Assessment Guidelines. *Regul Toxicol Pharmacol* **29**, 327-357.

Foster, P. M. D. (2005). Mode of action: impaired fetal leydig cell function--effects on male reproductive development produced by certain phthalate esters. *Crit Rev Toxicol* **35**, 713-9.

Foster, P. M. D. (2006). Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. *Int J Androl* **29**, 140-7; discussion 181-5.

Gray, L. E., Jr., Ostby, J., Furr, J., Price, M., Veeramachaneni, D. N., and Parks, L. (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male Rat. *Toxicol Sci* **58**, 350-65.

Howdeshell, K. L., Furr, J., Lambright, C. R., Rider, C. V., Wilson, V. S., and Gray, L. E., Jr. (2007). Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. *Toxicol Sci* **99**, 190-202.

IARC (2000). Monographs on the evaluation of carcinogenic risks to humans. Some industrial chemicals. . **77**, 41-148.

Ito, Y., Yamanoshita, O., Asaeda, N., Tagawa, Y., Lee, C. H., Aoyama, T., Ichihara, G., Furuhashi, K., Kamijima, M., Gonzalez, F. J., and Nakajima, T. (2007). Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *J Occup Health* **49**, 172-82.

Melnick, R. L. (2002). The IARC evaluation of di(2-ethylhexyl)phthalate (DEHP): a flawed decision based on an untested hypothesis. *Int J Occup Environ Health* **8**, 284-6.

Melnick, R. L., Brody, C., DiGangi, J., and Huff, J. (2003). The IARC evaluation of DEHP excludes key papers demonstrating carcinogenic effects. *Int J Occup Environ Health* **9**, 400-2.

Metzdorff, S. B., Dalgaard, M., Christiansen, S., Axelstad, M., Hass, U., Kiersgaard, M. K., Scholze, M., Kortenkamp, A., and Vinggaard, A. M. (2007). Dysgenesis and histological changes of genitals and perturbations of gene expression in male rats after in utero exposure to antiandrogen mixtures. *Toxicol Sci* **98**, 87-98.

Silva, M. J., Barr, D. B., Reidy, J. A., Malek, N. A., Hodge, C. C., Caudill, S. P., Brock, J. W., Needham, L. L., and Calafat, A. M. (2004a). Urinary Levels of Seven Phthalate Metabolites in the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Environ Health Perspect* **112**, 331-8.

Silva, M. J., Reidy, J. A., Herbert, A. R., Preau, J. L., Jr., Needham, L. L., and Calafat, A. M. (2004b). Detection of phthalate metabolites in human amniotic fluid. *Bull Environ Contam Toxicol* **72**, 1226-31.

Voss, C., Zerban, H., Bannasch, P., and Berger, M. R. (2005). Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology* **206**, 359-71.

Wilson, V. S., Howdeshell, K. L., Lambright, C. S., Furr, J., and Earl Gray, L., Jr. (2007). Differential expression of the phthalate syndrome in male Sprague-Dawley and Wistar rats after in utero DEHP exposure. *Toxicol Lett* **170**, 177-84.

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