BSC Research Concept Review

Meeting (December 6, 2007) of the National Toxicology Program Board of Scientific Counselors

Post- BSC Meeting Review
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NTP Study Nomination: Phthalate Initiative

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1) Is a clear and valid rationale for the proposed research program articulated in the NTP research concept document?

The proposed program has clear rationale that is based in part on recent findings (Ito et al, 2007) that lead to uncertainty over the role of the nuclear receptor PPARa in mediating the hepatocarcinogenic potential of DEHP in mice. Coupled with studies showing the impact of perinatal exposure to phthalates on other effects, there is a solid general basis for this program.

The proposal's first hypothesis is that the carcinogenic potential of DEHP in animals might be influenced by the developmental age at the start of treatment. Since the non-cancer effects of DEHP in rodents include reproductive and developmental toxicity, this hypothesis seems reasonable. In addition, there are currently no data available to address this issue.

In order to address the proposal's first hypothesis, a study of DEHP comparing start of exposure in utero vs. adult aged animals is proposed. The study design is appropriate, while the rationale for selection of the Wistar Han strain would presumably be further described. This is because among the references cited, Voss et al (2005) documents the use of the Sprague Dawley strain to characterize tumor response in liver and testis. In contrast, it is not certain that the Wistar Han strain would be similarly responsive to DEHP, even with a similar experimental design. This study would be potentially enhanced by additional interim endpoints that would address both PPARa-dependent and PPARa-independent mechanisms that are considered relevant to the development of tumors in liver and in other target tissues.

The proposal's second hypothesis is that PPARa is developmentally regulated and analysis of its expression could be used along with results of the perinatal exposure study to clarify mechanism. For example, it is assumed that characterization of PPARa expression of certain affected tissues in utero would enable the categorization of at least some of the effects of DEHP as PPARa-independent. This approach is somewhat less than definitive, as any increased risk of the development of neoplasms would be confounded by onset of expression during the perinatal exposure period. As such, the window for increasing risk of neoplasia may not be sufficiently defined to enable clarification of the role of PPARa.

The experimental strategy to proposal's second hypothesis would presumably need to be include other information that identifies the mechanisms for the effects in PPARa-negative tissues, although this is not addressed in the proposal.

It might be useful to briefly address the intended strategy (or strategies) for detecting receptor expression described under the second hypothesis of the proposal. One suggestion would be to expand the characterization to include expression of other PPAR isotypes (PPARg and PPAR b/d), since some phthalates and/or their metabolites can activate these isotypes. Another suggestion would be to use a variety of techniques that would allow an integrated characterization of expression. In addition, regional assessment at the level of tissue substructure and distinct cell populations is recommended. Finally, the rat strain should be shifted to Sprague Dawley, if that is the strain used in the perinatal study.

The proposal's third hypothesis is that risk of lifetime (including perinatal) exposures to phthalate mixtures would be additive of individual phthalates for cancer outcomes. The proposed research very appropriately recognizes and addresses the need to establish toxicokinetic parameters with particular attention to route of administration (oral/dietary route of administration is suggested upon review). However, the selection of appropriate biomarkers is somewhat challenging, as their utility might be limited by relevant correlation across tissues evaluated and by the uncertain nature of their relationship to mechanism of action.

For the third hypothesis, some additional discussion of the strategy of selection of phthalates would be useful. For example, based on listed references on rodent carcinogenicity studies, it appears that 2 of the named phthalates (DEHP and DINP) have been associated with liver tumors and 2 of the named phthalates (DEHP and DBP) have been associated with testis tumors. It is possible but not clear whether additional data from rodent carcinogenicity studies exist and could add to the already cited effects of other phthalates, in order to inform the design of the proposed work.

> *2) Is the proposed research program as outlined in the research concept document appropriate in scope given the public health importance of the issue or substance proposed for study? Are there other studies that should be considered as part of this research program?*

Phthalates are present as environmental contaminants and in consumer products, and there is significant public concern. This proposal to characterize the potential health effects of phthalates, particularly with respect to perinatal exposure, will likely impact public health decisions concerning phthalates.

If there is an opportunity to expand this program, it would be in the area of further delineating the PPARa-dependent from the PPARa-independent effects of the phthalates. This is addressed in limited fashion in the existing proposal by the ontogeny study of PPARa expression in the rat with the aim of relating receptor expression to target tissue effects in perinatal studies. However, there is a significant opportunity to more directly address the carcinogenic effects of phthalates and their underlying mechanisms (at least in liver) in studies of PPARa knockout

mice. In addition to use of the PPARa knockout mice, further studies could be extended to include the humanized PPARa mice (knock-out mouse gene plus knock-in human gene). While the efforts needed to conduct this work in well-designed studies are not trivial, they are: 1) almost uniquely aligned to the capability of NTP to execute highly complex studies in an unambiguous manner, 2) clearly relevant to characterizing the effects of phthalates and likely to yield the most useful information for understanding the role of PPARa (where the level of uncertainty has increased, as suggested by the data of Ito et al).

3) *Does the proposed research program address an important area of biomedical research (e.g. children's health, genetic susceptibility, specific environmental disease) and/or advance the field of environmental health sciences?*

The proposed research program would be most pertinent to understanding the effects of perinatal exposure. This is partly but not entirely related to children's health, since there is the possibility that perinatal exposure can contribute differently to effects (such as cancer) that are seen later in adult life. The proposed research program also begins to address the characterization of effects of phthalate mixtures and thus begins to inform the assessment of more complex exposure scenarios.

4) *Does the proposed research program merit utilization of NTP resources, and if so, what priority (low, moderate, or high) should it be given?*

The proposed research program should be considered for a high priority. A tiered approach should be considered, as the value and significance of the proposed work under hypotheses 2 and 3 are highly dependent upon the results of the carcinogen bioassay study of perinatal exposure under hypothesis 1. If the carcinogen bioassay study of perinatal exposure under hypothesis 1 does not indicate any altered risk (as compared to adult onset of exposure), the proposed work under hypotheses 2 and 3 are considered low priority.

There are additional investigational approaches that are not described within this program proposal, but are highly relevant to its overall objectives. These additional approaches clearly merit consideration by the NTP. There is recent evidence (Ito et al, 2007) that increases the uncertainty over the relative roles of PPARa-dependent and PPARa-independent mechanisms in the hepatocarcinogenicity of one of the phthalates, DEHP. Some opportunities to maximize the impact of this program would include the addition of mechanistic investigation to the proposed perinatal study of DEHP in the rat (strain selection to be determined). A further, significant opportunity would be to expand the characterization of PPARa-dependent and PPARa-independent mechanisms for phthalates using the PPARa knockout mouse and the humanized PPARa mouse.

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