Challenges in the Application of Quantitative Approaches in Risk Assessment: A Case Study with DEHP

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Objective: To conduct a risk assessment using innovative techniques that allow for the incorporation of quantitative information associated with key events in the mode of action of a compound acting via a receptor-mediated mechanism.
Project Plan

- **Compound Selection**
  - a potential human health concern for both cancer and noncancer effects and “data rich” (well studied in animals, limited data in humans).

- *It was not a “goal” to find “the number” but to investigate the potential impact and the potential for use of innovative quantitative techniques in risk assessment.*
Project Plan

- **Standard Dose-Response Assessment**
  - NOAEL/LOAEL
  - BMDS

- **Incorporation of Advanced Approaches**
  - PBPK Model
  - Precursors
  - Genomics

- **Exposure Assessment**
  - Biomonitoring Results

- **Risk Characterization**
  - Comparison of internal blood concentrations
  - Uncertainty Analysis
    - Monte Carlo or Bayesian techniques
    - Impact of scientific judgment (Clewell et al. 2008)
    - Decision tree diagram (Clewell et al. 2008)
Selection of DEHP for Case Study

- Phthalates are in a wide variety of consumer products, widespread human exposure.
- “Data rich”
  - Well-studied in animals, limited data in humans.
- Effects well-characterized and the potential modes of action for some endpoints have been hypothesized (e.g., receptor-mediated).
- Phthalate PBPK models are available (Keys 1999; Clewell et al. 2008).
- Biomonitoring data are available (NHANES)
Recent studies (2002-present) that allowed for the comparison of effects across:

- a wide range of doses, in particular in the low-dose range;
- a wide range of exposure periods to include gestation, lactation and postnatal;
- several routes of exposure;
- multigenerational studies; and
- cancer and noncancer endpoints.
Reproductive/Developmental Studies

- **Andrade and Colleagues (gavage)**
  - administered 10 doses ranging from 0.015 to 405 mg/kg/day DEHP from GD6 to PND21, and evaluated effects in male and female offspring of Wistar rats. Grande et al. 2006; Andrade et al. 2006a; Andrade et al. 2006b; Andrade et al. 2006c; Grande et al. 2007).

- **Akingbemi and Colleagues (gavage)**
  - administered 6 doses ranging from 10 to 750 mg/kg/day for various intervals from PND 21 to 120, and evaluated effects on Leydig cell androgen and estradiol biosynthesis in male Long Evans rats (Akingbemi et al. 2001; Akingbemi et al. 2004; Ge et al. 2007).
Reproductive/Developmental Studies

- **National Toxicology Program (Dietary)**
  - Complex, three generation continuous breeding study in Sprague-Dawley rats (NTP 2004)

- **Kurahashi and Colleagues (inhalation)**
  - Prepubertal effects in male rats (Kurahashi et al. 2005) and in female rats (Ma et al. 2006).
Cancer Studies

- **National Toxicology Program (diet)**
  - Administered diets to male and female rats and mice containing 3000 to 12000 ppm DEHP for lifetime

- **David and Colleagues (diet)**
  - Administered diets to male and female B6C3F1 mice and Fischer 344 rats containing 100 to 12500 ppm (David et al. 1999, 2000a,b) DEHP for 104 to 159 weeks.

- **Voss and Colleagues (diet)**
  - Administered diets to male and female Sprague-Dawley rats containing 30 to 300 mg/kg/day DEHP for 159 weeks.
Endpoints - Noncancer

- Age at vaginal opening (females)/age at preputial separation (males)
- Aromatase activity in the brain (males/females)
- Anogenital distance (males)
- Bi- and multi-nucleated gonocytes (males)
- Serum and testicular interstitial testosterone (males)
- Sperm Production
- Sperm morphology
- Serum LH concentrations
- Leydig cell production
- Steroidogenic enzyme activity
- E2 concentrations
- Age at first estrous
Endpoints - Cancer

- Hepatocellular adenomas/carcinomas
- Leydig cell tumors
- Pancreatic acinar cell tumors
NOAEL/LOAEL approach

- Initial “baseline” approach for comparison to Points of Departure (PODs) following incorporation of other approaches.

- Depending on the results of statistical analyses to make decisions regarding the POD could result in values ranging from 0.015 to 405 mg/kg/day, depending upon the scientific judgment used in selecting an “adverse” effect.
NOAEL/LOAEL approach

- **Possible oral PODs**
  - 0.015 mg/kg/day – significant *increase* in anogenital distance (significant decrease observed at 405 mg/kg/day)
  - 0.045 mg/kg/day – significant increase in serum testosterone and decrease in sperm production
    - Historical control comparisons (1.215 mg/kg/day)
    - Biological significance
      - Sperm production decrease of 20% (5 mg/kg/day)
      - Impact on reproductive performance (405 mg/kg/day)
  - 15 mg/kg/day – significant dose-related changes for endpoints associated with onset of puberty
Possible inhalation PODs

- Exposures limited to concentrations of 5 or 25 mg/m$^3$
- Duration of exposure was critical for effects (4 weeks versus 8 weeks) suggesting a possible critical window of exposure
- Lowest concentration administered was a LOAEC, based on decrease in age of vaginal opening and age at first estrous in female rats.
Benchmark Dose Modeling

- Takes into account all the dose-response information.
- Can remove some “all” or “nothing” decisions (i.e., determining whether or not a NOAEL was a defined at a particular dose.
- Considers uncertainty with smaller sample size (i.e., wider confidence limits).
- Can identify a NOAEL equivalent when a NOAEL has not been identified in the study.
Andrade et al. (2006a)
Andrade et al. (2006c)
Ge et al. (2007)

Accumulative Frequency of preputial separation (%)

PND (days)

0 mg/kg DEHP

10 mg/kg DEHP

500 mg/kg DEHP

750 mg/kg DEHP
Because of the nonmonotonic nature of the majority of the responses, an adequate fit ($p>0.05$) of the models in BMDS could not be achieved. Those datasets for which an adequate fit would be achieved only demonstrated significant responses on the upper end (>10 mg/kg/day) of the dose-response information. Measures were attempted to achieve an adequate fit (e.g., dropping both high and low doses).
Kurahashi et al 2005 – Increased seminal vesicle weight Testosterone (8 weeks) - Constrained

Polynomial Model with 0.95 Confidence Level

Polynomial

BMD

BMDL

dose
Kurahashi et al 2005 – Increased seminal vesicle weight Testosterone (8 weeks) - Unconstrained

Polynomial Model with 0.95 Confidence Level
Endpoints providing evidence that a particular window of exposure may be the most sensitive period lacked a conventional monotonic dose-response.

Application of BMD modeling was not adequate to address these changes, even using nonconventional methods (i.e., dropping dose groups, smoothing of dose-response information)

However, this is not justification to exclude these data from consideration, rather should challenge the risk assessor to understand the biological implications of these changes.
Cancer Dose-Response Modeling

- Consideration of weight of evidence and relevance to human health for cancer endpoints could result one of two classifications for DEHP
  - “Likely to be carcinogenic to humans” (pancreatic acinar cell, Leydig cell tumors)
  - “Not likely to be carcinogenic” – PPARα agonists

- Dose-response modeling was conducted for all endpoints for comparison.
Cancer Dose-Response Modeling

- **Possible animal PODs**
  - 52 mg/kg/day – liver adenomas and carcinomas in female mice (David et al. 1999)
  - 97 mg/kg/day – Leydig cell tumors in male rats (Voss et al. 2005)
The metabolite, MEHP, or some of MEHP’s downstream oxidative metabolites have been suggested to be associated with adverse testicular effects (ATSDR 2002; Koch et al. 2005).

Investigated multiple dose metrics
- Gestational - AUC maternal blood, fetal blood, fetal testes
- Lactational – AUC maternal blood, pup blood
- Postnatal – AUC blood

Incorporation of dose metrics did not significantly improve BMD model fit for noncancer endpoints.
Incorporation of dose metrics did not significantly improve BMD model fit for noncancer endpoints.

While adequate fits were obtained for cancer endpoints, comparison of the use of external dose to the internal dose metrics considered did not significantly improve model fit.

Route-to-route comparison (based on blood concentrations)
- 5 mg/m³ comparable to 1 mg/kg/day via gavage
- 25 mg/m³ comparable to 10 mg/kg/day
Integration of Data

  - suggest an expansion of data to be considered as part of the risk assessment process.
  - unify/harmonize cancer and noncancer endpoints
  - assessment of background disease processes and exposures, possible vulnerable populations, and modes of action that may affect a chemical’s dose-response relationship in humans (NAS 2009).
Integration of Data

- **NAS (2008)**
  - *On Phthalates and Cumulative Risk Assessment*, the committee strongly recommends that EPA group chemicals that cause common adverse outcomes and not focus exclusively on structural similarity or on similar mechanisms of action.
Integration of Noncancer effects by Administered Dose of DEHP

<table>
<thead>
<tr>
<th>Route and Duration</th>
<th>Effects at Doses of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.015 to 10 mg/kg/day</td>
</tr>
<tr>
<td>Gavage</td>
<td>anogenital distance ↑</td>
</tr>
<tr>
<td></td>
<td>sperm production ↓</td>
</tr>
<tr>
<td></td>
<td>testes weight ↑</td>
</tr>
<tr>
<td></td>
<td>---</td>
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<td></td>
<td>---</td>
</tr>
<tr>
<td>Gavage and Inhalation</td>
<td>male testosterone levels Δ</td>
</tr>
<tr>
<td>(greater than 20 days postnatal)</td>
<td>Leydig cell proliferation ↑</td>
</tr>
<tr>
<td></td>
<td>seminal vesicle weight ↑</td>
</tr>
<tr>
<td></td>
<td>onset of puberty ↑</td>
</tr>
<tr>
<td>Dietary</td>
<td>---</td>
</tr>
<tr>
<td>(multigen)</td>
<td>F2 onset of puberty ↓</td>
</tr>
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<td></td>
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</tbody>
</table>

Increase in Testosterone  Decrease in Testosterone
Comparison of NOAEL/LOAEL

Dashed lines indicate less confidence and bolder lines indicate greater confidence. Broader solid lines identify the preferred POD based on confidence.
Comparison of BMDLs for Cancer

Dashed lines indicate less confidence and bolder lines indicate greater confidence.
Integration of PODs for Noncancer and Cancer
Integration of PODs for cancer and noncancer and consideration of confidence and relevance to human health suggests a POD of 50 mg/kg/day associated with the multi-generational dietary study (NTP 2004).
PBPK model and NHANES biomonitoring data for DEHP metabolites

- provides risk assessor with an opportunity to compare a POD to an external concentration associated with measured urine concentrations in the general population
- By including Monte Carlo approaches that consider distributions of values rather than point estimates in the PBPK model, a distribution of intakes for the general population associates with the range of measured urine concentrations is possible.
Exposure Assessment

- **NHANES results for primary metabolite (MEHP)**
  - 4.1 ng/ml (median)
  - 38.9 ng/ml (95th percentile)

- **Secondary metabolites**
  - 14.0 to 20.1 ng/ml (median)
  - 120 to 192 ng/ml (95th percentile)

- **Reverse dosimetry**
  - 1.45 µg/kg/day (median)
  - 0.13 and 20.4 µg/kg/day (5th and 95th percentiles)
Comparison of the lowest PODs to results of reverse dosimetry

- Noncancer MOE - 50 mg/kg/day /0.13 or 20.4 µg/kg/day for a resulting MOE of 2500 (95\textsuperscript{th} percentile) to 27000 (median)
- Cancer MOE – 97 mg/kg/day/0.13 or 20.4 µg/kg/day for a resulting MOE of 4800 (95\textsuperscript{th} percentile) to 52000 (median)

Outside the application of Uncertainty Factors, these MOEs can still provide information to inform a risk assessor in making decisions regarding the potential for human adverse effects.
Lessons Learned

- The intent of this case study was not to develop “the number” for DEHP, but evaluate the incorporation of new quantitative methods into the standard risk assessment process.

- Mathematical models are not always adequate for dose-response assessments.

- Integration of all the available toxicological data is critical to understanding the biological processes being impacted
  - Review of individual studies and individual datasets can be misleading.
  - Integration across studies can provide a form of “biologically based dose-response” assessment without the incorporation of complex mathematical approaches.
Lessons Learned

- Consideration of those routes of exposure relevant to human populations must be given highest priority.

- It is likely that the issues encountered as part of this exercise are not limited to DEHP, but also important for other receptor-mediated compounds or compounds that “perturb” biological pathways, such as endocrine disruptors.
Lessons Learned

- The approach applied doesn’t require the demonstration of a mode of action to justify the shape of the low-dose region or a threshold, but integrates the data in a manner that the shape of the dose-response curve is described by all of the available data.
Lessons Learned

- Highlights the need for researchers and risk assessors to communicate and focus on critical questions
  - What are the residual concerns?
  - What are the major data gaps?
  - What studies are needed?
  - What research will best inform the next risk assessment conducted for this compound?
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Announcement

Building for Better Decisions: Multi-scale Integration of Human Health and Environmental Data

WHY: Toxicologists now face the challenge of integrating increasingly complex data to translate our work and predict adverse outcomes in various applications.

WHAT: An SOT Contemporary Concepts in Toxicology Workshop, an open, international conference featuring breakout groups related to exposure, dose-response, ecosystem impacts, life cycle/cost-benefit, and information technology that provide an opportunity for scientific discussion and debate. In addition, abstracts can be submitted for poster presentations to facilitate discussions and develop recommendations.

WHO: Sponsored by the SOT and US EPA with:
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- SRA
- ISES
- EMS
- ACC LRI
- Environ Corp.
- OGC
- OpenMI
- TERA

WHEN: May 8 – 11, 2012

WHERE: US EPA, RTP Campus, NC

For complete information (Agenda, Abstract submission, Registration, etc) go to: https://www.toxicology.org/ai/meet/cct_b4bd.asp

Deadline for Abstract Submission is March 23, 2012