ITER Peer Review on Butyl Benzyl Phthalate, Lead & Arsenic Meeting Summary

April 27, 1998
University of Cincinnati, College of Medicine
Cincinnati, Ohio USA

An independent panel of expert scientists and risk assessors met on April 27 to review an assessment of butyl benzyl phthalate and a research program for measuring bioaccessibility of lead and arsenic in contaminated soils. This meeting was conducted by Toxicology Excellence for Risk Assessment (*TERA*); a non-profit organization dedicated to the best use of toxicity data in risk assessment. Expert peer reviewers donated their time and talents to provide an independent review of the assessment and research program. A comprehensive overall review of the materials was provided by the combined experience of all the reviewers.

The peer review meeting began with a discussion of conflict of interest. Prior to the meeting each reviewer certified that he or she did not have a conflict (real or apparent) with the chemical under review or sponsor, or identified the potential for such conflicts. Possible conflicts were discussed with each reviewer to determine if measures were needed to manage a potential conflict (or appearance of conflict). Options include excluding the reviewer from a particular discussion and consensus, or allowing the reviewer to participate in the discussion, but not be polled for consensus. The peer review panel discussed and agreed upon how to manage any potential conflicts. This is documented in Attachment A.

These review meetings follow a standard format beginning with a close examination of the supporting documentation and important references several weeks prior to the meeting. At the meeting, after the conflict of interest discussion and decision by the panel is made, the authors of the assessment or documentation briefly present their work. For chemical assessments, the panel then systematically discusses the assessment, starting with a determination of whether adequate data exist on which to base a risk value, followed by a discussion of the appropriate critical endpoint and study. Next, the quantitative aspects of the assessment are discussed.

For the bioavailability research program discussion centered on a list of issues and questions identified by the sponsor, with the reviewers providing additional issues. Full discussion and participation are encouraged and agreement is reached by consensus. Consensus for the purpose of these meetings is defined as "an opinion held by all or most, or general agreement."

The meeting was open to the public with several observers from industry, the U.S. Environmental Protection Agency, Colorado University, and the University of Cincinnati.

Butyl Benzyl Phthalate Assessment

Sponsor: Health Canada
Presenters: Mr. George Long and Ms. Bette Meek, Health Canada
Chair: Dr. Michael Dourson, *TERA*

Review Panel:

- Dr. Mohamed S. Abdel-Rahman, New Jersey Medical School,
- Dr. John P. Christopher, California Environmental Protection Agency (Cal EPA)
- Dr. George P. Daston, Procter and Gamble Company
- Dr. Joyce M. Donohue, U.S. EPA, Office of Water
- Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment (*TERA)*
- Ms. Deborah Proctor, ChemRisk Division of McLaren/Hart
- Ms. Ruthann Rudel*, Silent Spring Institute
- Dr. Alan H. Stern, New Jersey, Department of Environmental Protection

* Provided written comments for the panel's consideration.

PRESENTATION & CLARIFYING QUESTIONS

Health Canada proposed a Tolerable Daily Intake (TDI) in their document on butyl benzyl phthalate, which was prepared as part of the Priority Substances Program mandated under the Canadian Environmental Protection Act. Mr. George Long and Ms. Bette Meek of Health Canada provided a brief summary of the toxicological basis for the proposed TDI and a background on the prior reviews of the documentation.

Butyl benzyl phthalate (BBP) is a plasticizer used in polyvinyl chloride and other polymer products. The primary route of exposure is thought to be through ingestion of food. There is little data on the health risks of BBP in humans; however, toxicity has been well characterized in a range of recent toxicity studies in animals, primarily by the oral route. A TDI of 1.3 mg/kg b.w./day was proposed based on the critical effect of pancreatic lesions observed in a subchronic dietary study in Wistar rats (Hammond et al. 1978). The 95% lower confidence limit of the estimated benchmark dose (BMD_{05}) of 132 mg/kg b.w./day was used in conjunction with a total uncertainty factor of 100 (10 for interspecies variation x 10 for intraspecies variation) to derive this value. It was acknowledged that the proposed TDI represents a "snapshot in time" and while further studies which are underway are noted in the documentation, mandated timeframes for delivery of assessments for all Priority Substances under the Canadian Environmental Protection Act necessitate closure on the TDI at this time.

Clarifying Questions on the Presentation:

Following the presentation, the panel asked clarifying questions. Clarification was requested on the status of the three low-dose *in utero* exposure studies: Sharpe et al. (1995), Ashby et al. (1997), and TNO (1997), and whether there was any *in vivo* evidence

for the estrogenicity of BBP. The resulting discussion on reproductive and endocrine effects is summarized in the hazard identification section. The issue of purity of the BBP used in the experiments was raised, as well as the impact of the use of plastic versus glass containers on delivered doses for the low-dose studies. An observer from Solutia (the manufacturer of BBP and sponsor of several studies) volunteered that BBP is generally 98% BBP with a small amount of monoesters present. BBP can be absorbed by plastic due to its hydrophobic nature, which was the rationale for using glass and stainless steel in the TNO (1998) study. There was also some discussion concerning whether differences in toxicological effects in rats and mice might be attributable to differences in metabolism, although the limited available data were considered inadequate to draw conclusions in this regard.

DISCUSSION

Hazard Identification

The panel agreed that the existing database was sufficient for development of a TDI.

The panel reviewed the choice of pancreatic lesions observed in a 90-day dietary study in Wistar rats (Hammond et al. 1987) as the critical effect. The relevance of kidney and liver weight changes observed in several animal studies was discussed and general agreement was reached that these changes were not particularly convincing as critical effects in the absence of corroborating histopathological changes. Health Canada stated at the meeting (and in the supporting documentation) that even if these effects were assumed adverse, their use would result in a similar TDI estimate. In written comments, one reviewer raised the question of the liver weight changes observed at very low doses following *in utero* exposure reported in Ashby et al. (1997). Health Canada pointed out that this result has not been verified by other independent studies. Panel members felt that without evidence that these effects generate tissue lesions their use would not be appropriate to define the critical effect. Health Canada agreed that the observation of increased liver weight in the offspring in the Ashby study should be included in the discussion (in the Hazard Evaluation and Dose-Response Analyses section of the document) where they indicate why increases in organ weights in the absence of histopathological change are dismissed as the critical effects.

The panel discussed why the kidney lesions observed in the 14-day reproductive study (Agarwal et al. 1985) in Fisher rats and those observed in the NTP (1997) chronic bioassay were not selected as the critical effect: the LOAEL for this effect is lower than that observed for the pancreatic lesions. Health Canada considered this in their documentation; however, a benchmark dose estimate generated for this effect in the reproductive study generated a poor fit compared to the pancreatic lesion data in the subchronic bioassay. In addition, the high incidence of renal lesions observed in the NTP (1997) chronic bioassay in all the dose groups had no clear dose-related increase. Another reviewer commented that renal effects are common in rats, even in the absence of exposure, and therefore the rat is not a good model for evaluation of renal lesions. Health

Canada agreed to add to their documentation the benchmark dose analysis for renal lesions in the NTP study which demonstrated poor dose-response and goodness of fit.

One reviewer noted that the nature of the pancreatic lesions that were observed in the Hammond et al. (1987) and NTP (1997) studies were not the same. A panel member suggested that the pancreatic effects observed in the NTP study may provide a better estimate of the TDI due to a possible better quantification of the doses used in this study. The problem of uncertainty in classification of hyperplasia versus adenoma could be reconciled by combining these effects for additional benchmark dose analysis. Another reviewer pointed out that it might be prudent to use the effects observed in the subchronic bioassay if they represent precursors to the hyperplasia and adenoma reported in the chronic bioassay. Health Canada agreed that this analysis of the data from the chronic study might be useful to strengthen their case for pancreatic lesions as the critical effect. (NOTE: Subsequent to the meeting it was determined that the quantification of the NTP study doses was not better, and due to the nature of the reporting in the NTP study, it is not possible to conduct an analysis based on hyperplasia and adenomas combined.)

Much of the review panel discussion centered around the issue of effects on male reproductive organs. Several studies reported effects on the testes; however, these effects generally occurred at doses higher than those associated with effects on the pancreas (Hammond et al. 1987). The panel discussed why these effects were observed in short-term but not the chronic studies, with one person noting that this might be explained by the masking effect of frank toxicity observed at chronic doses high enough to generate testicular effects. The panel was asked to consider whether the choice of pancreatic effects would protect against the potential endocrine-mediated effects of BBP reported at very low doses in Sharpe et al. (1995). Health Canada indicated that three recent studies have evaluated the effect of BBP on the testes following *in utero* exposure, but the results have been inconsistent and the TNO (1997) study includes only preliminary results and cannot be weighted heavily. One reviewer indicated that the Ashby et al. (1997) study and current work by Sharpe have failed to corroborate the initial low dose effects on the testes reported in the published report by Sharpe et al. (1995).

The panel discussed the possible endocrine and reproductive effects, including the fact that there are no *in vivo* data to support the idea that BBP is estrogenic, although the yeast data indicate weak activity. One possible explanation for this that was noted is that unlike *in vivo*, no metabolism of BBP to the monoester occurs *in vitro*. The relevance of the existing toxicity data on dibutyl phthalate (DBP) was discussed since both compounds may be metabolized to the monoester. Health Canada asked for specific input from the panel on the text in the supporting document on the endocrine effects of BBP. In response, one panel member suggested that the text make clear that BBP is generally not believed to be estrogenic; however, this does not preclude other potential endocrine mediated effects. As an example, the reviewer discussed DBP, which is questionably estrogenic. However, recent work indicates that DBP may act through other hormone-mediated mechanisms including anti-androgenic effects. The dose of DBP required to generate these effects is similar to those that generate other effects of toxicity, therefore,

in analogy to DBP, similar endocrine effects by BBP would not necessarily change the critical effect level.

The panel agreed that the pancreatic lesions were the appropriate choice for a critical effect, while noting that potential endocrine effects at low doses are an issue in need of further study.

Dose-Response Assessment

Choice of Dose: The panel discussed the calculation of the benchmark dose based on the data from the Hammond et al. (1987) study in which a dose-dependent increase in pancreatic lesions was observed following exposure to BBP in the diet for 90 days.

A reviewer raised a question about the potential confounding effect of the observed difference in pharmacokinetics of BBP at high versus low doses. At least in one study, the available data indicate that at high doses a greater percentage of administered BBP was excreted in feces, a potential indication that saturation of BBP absorption occurs. The panel discussed the implications for interpretation of effects observed at the high doses in critical animal studies. Another reviewer commented that in the worst case flattening of the dose response curve might be generated at high doses. However, another reviewer pointed out that even if this effect occurs, enough absorption would occur at high doses to generate the same effects observed at the lower doses. It was suggested that an approach to test whether this effect alters dose-response at low doses would be to compress the data from the two high dose groups and refit the dose-response data to the BMD model. This could be done for both the pancreatic lesion data since this is the critical effect, and the kidney lesion data, in order to provide a study with doses both below and within proximity to those at which saturation of absorption may occur. (NOTE – Subsequent to the meeting, Health Canada did combine the top two does and it had minimal effect on the quantitative value.)

Several reviewers noted that the graphical representation of the BMD model did not appear to be a standard mathematical function; rather, it appeared to be very data specific. They asked for additional details on the choice of the model for the BMD analysis in order to evaluate the appropriateness of the results. Other panel members expressed satisfaction with the model and one reviewer commented that the model results were not unexpected since at high doses one would expect to see a flattening out of the response curves. It was suggested that Health Canada consider providing greater description of the choice of BMD models and additional details including, actual data and confidence bars, maximum likelihood estimates, and limits of BMD regression.

Uncertainty Factors: Health Canada proposed a total uncertainty factor of 100, with 10 to account for interspecies variation and 10 for intraspecies variation.

Intra- and Inter- Species: The panel agreed that the data do not support departure from the default uncertainty factors of 10 for interspecies and intraspecies variation. It was

suggested that Health Canada explicitly state that the data were insufficient to replace the defaults for kinetic and dynamic components with data-derived values.

Data Deficiencies: Most of the uncertainty factor discussion centered on the need for additional factors. In response to a question from the panel, Health Canada explained that under their procedures an uncertainty factor for data base adequacy can be applied to ensure that the TDI accounts for additional more sensitive endpoints. In this case, the existing data on potential endocrine effects of BBP are too weak to warrant an additional factor. One panelist indicated that perhaps a factor should be applied for this endpoint in light of uncertainty surrounding this effect. However, another panel member indicated that due to the tenuous nature of the data on this effect and the existing data for DBP, this factor might not be needed.

One panel member suggested the addition of an uncertainty factor (perhaps 3) for potential exposure to multiple phthalates since the Canadian methodology (Meek et al. 1994) allows for this consideration. Health Canada indicated that this would be difficult to incorporate quantitatively and more detailed exposure data would be needed. In addition, TDIs have been established for other similar phthalates. Another reviewer suggested that additional analysis of the BMD model might make it appropriate to add an additional factor to consider inadequacy of the model to account for the effects of absorption differences at high doses.

Although individual reviewers initially suggested additional uncertainty factors as described above, the panel reached consensus on the proposed 100 uncertainty factor.

The panel also reached consensus on the proposed TDI of 1.3 mg/kg b.w./day. Some members suggested that the precision of the proposed TDI should be changed to one significant digit. Health Canada responded that though they agreed with the intent of the comment, the suggestion would need to be considered in the context of internal consistency of their TDIs.

RECOMMENDATIONS

- Investigate the utility of additional analyses of the data from the chronic study (NTP 1997) as it might be useful to strengthen the case for pancreatic lesions as the critical effect.
- Consider providing greater description of the choice of BMD models and provide additional details, including actual data and confidence bars, maximum likelihood estimates, and limits of BMD regression.
- State explicitly that the kinetic and dynamic data were insufficient to replace the default uncertainty factors for either intraspecies or interspecies extrapolation.

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Research Program for an *In vitro* Method for Measuring Bioavailability of Lead and Arsenic in Soils

Sponsor: U.S. EPA Region 8 on behalf of the Solubility/Bioavailability Research Consortium (SBRC)
Presenters: Dr. Gerry Henningsen, U.S. EPA Region 8 and Mr. Michael Ruby, Exponent
Chair: Dr. Michael Dourson, *TERA*

Review Panel:

- Dr. Mohamed S. Abdel-Rahman, New Jersey Medical School,
- Dr. John P. Christopher, California Environmental Protection Agency (Cal EPA)
- Dr. Joyce M. Donohue, U.S. EPA, Office of Water
- Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment (*TERA)*
- Dr. Fran V. Kremer, U.S. EPA, Office of Research and Development

- Ms. M.E. (Bette) Meek, Health Canada
- Dr. Rashmi S. Nair, Solutia
- Ms. Deborah Proctor, ChemRisk Division of McLaren/Hart
- Ms. Sandy M. Roda, University of Cincinnati
- Dr. Alan H. Stern, New Jersey, Department of Environmental Protection
- Dr. Paul A. Succop, University of Cincinnati

Representatives of the Solubility/Bioavailability Research Consortium (SBRC) presented information on the *in vivo* and *in vitro* work of this project. This was followed by a short period during which reviewers asked clarifying questions. The panel then discussed the questions and issues which made up their charge.

PRESENTATION & CLARIFYING QUESTIONS

Dr. Gerry Henningsen, U.S. EPA Region 8

Dr. Henningsen briefly presented information on EPA's *in vivo* bioavailability study results for lead and arsenic in soils and their applicability for *in vitro* bioaccessibility assays. He noted that EPA encourages site specific data in their risk assessments. This includes accurate estimates of bioavailability for use in the IEUBK (Integrated Exposure Uptake and Biokinetics) model for use in risk assessments on hazards to children from soil-lead.

To overcome some of the limitations of existing studies EPA has used juvenile pigs as models for young children, lower doses in the range ingested by children, and soil samples that are representative in composition and consistency to those contacted by children. Relative bioavailability (RBA) of lead was measured on 20 test materials (soils from Superfund sites). RBA was defined as a "ratio of doses" for any given non-linear response. Among the results were:

* good time- and dose-response was achieved;
* the model characterized a range of RBAs;
* blood kinetics were non-linear,
* tissues were linear;
* quantitative variability and uncertainty were described for the RBAs; and
* the quality assurance/quality control showed good accuracy, precision, and reproducibility.

Absorption of soil-lead is highly variable and is dependent on the physical-chemical nature of the lead particles and matrix. Semi-quantitative RBAs can be estimated for soil-lead, which can be presented as ranges of RBAs to risk managers to provide a range of uncertainty.

The EPA work with arsenic was conducted as a secondary effort to take advantage of the availability of soil samples and the work that was being done with the lead in juvenile

pigs. Preliminary "model characterization" results have many limitations but suggest an apparently lower range of RBAs for arsenic from soil.

The *in vitro* assays are desired to reduce the need for animal studies and are less costly and time consuming. Reasonable predictors of *in vivo* results are needed to attain broad RBA application for lead and arsenic. Solid *in vivo* correlations are an essential component of validating the *in vitro* assays. Proper geochemical speciation is critical for accurate application of results to other sites.

Michael Ruby, Exponent

Mr. Ruby described the work of the SBRC. The group began in January 1997 and includes members from EPA Region VIII; Massachusetts DEP; New Jersey DEP; Dupont; Exxon Biomedical; FMC Corp; Elf Atochem; University of Colorado, Boulder; University of Colorado, Denver; Exponent and ISSI Inc. Industry members have funded the *in vitro* work to date, with EPA Region 8 paying for the peer review and for all 20 *in vivo* studies employed in the comparison. The *in vitro* test is designed to estimate the Relative Absorption Fraction (RAF) which is absorption from the exposure medium of concern divided by absorption from the medium used in the toxicity study upon which the risk assessment is based.

Phase 1 of the project involved the development of a simplified *in vitro* test and included a sensitivity analysis of variables such as temperature, buffer concentration and fluid pH. A simplified protocol (SOP 1) for the test was developed and was one of the documents reviewed at this meeting. The method involves a buffered simulated stomach solution with 1 gram of soil added to 100 ml of fluid. The test material in solution is rotated end over end for one hour maintaining a temperature of 36 degrees centigrade. It is filtered and analyzed for lead and arsenic.

Preliminary results indicate that the test works well for lead (correlates with *in vivo* studies) and that the method is ready for formal validation. Investigators have taken advantage of the existing samples to see if the method would work with arsenic, but are not as far along as they are for lead. So far, however, the method has not been as predictive for arsenic.

Phase II of the *in vitro* correlation project involves inter-laboratory testing and simultaneous method validation using substrates which have been tested in *in vivo* assays. SOP 2 describes how to analyze the sample extracts from the simplified *in vitro* bioaccessibility method. The Quality Assurance Program Plan (QAPP) and SOP 2 are also being reviewed for adequacy to meet the program's goals. For the inter-laboratory validation study, four labs will analyze a set of 36 substrates according to the *in vitro* protocol. Results will be evaluated to assess method reproducibility and correlation to *in vivo* estimates of lead and arsenic bioavailability.

For 1998 the SBRC will conduct the validation study and hopes to further investigate methods for arsenic, including the importance of the small intestine phase and sensitivity

analysis on the test parameters. In addition, lead in paint and household dust samples will be evaluated.

Clarifying Questions on the Presentations:

The reviewers asked questions to clarify a number of issues. The two presenters, along with Dr. Christopher Weis of U.S. EPA Region 8 and Dr. John Drexler of Colorado University, answered the panel's questions.

One reviewer asked whether the pH of swine gastric juice is the same as in children. The sponsors indicated that hydrogen ion concentration during fed and fasted states varies widely by 4-5 orders of magnitude in swine, but this is similar to human variability.

A reviewer asked what forms the basis for the ratio of soil to acid for the extraction test. The sponsors responded that they created a system with a buffer strength designed to overwhelm the buffering capacity of the soil.

One reviewer noted that the IEUBK model uses an assumption that, on an absolute absorption basis, 30% of the ingested lead will be absorbed into the systemic circulation and will be bioavailable. She asked whether the *in vitro* results would be used to replace this 30% figure. The sponsors indicated that the IEUBK model uses an absolute measure of absorption of 30% for lead in soil and 50% for lead in water. Thus, the relative absorption of soil lead is 30/50, or 60%. Site-specific *in vivo* or *in vitro* studies can be used to similarly adjust relative lead absorption estimates if designed and implemented properly.

One reviewer noted that the two presenters used different definitions for relative bioavailability. The sponsors noted that the relative bioavailability definition is based on the ratio of doses and best defines the *in vivo* data. Relative bioaccessibility was defined as the ratio of solubilities (fractional solubility of mass), which is a ratio of the fraction of ingested lead or arsenic available for absorption.

Another reviewer questioned the proportion of clay or organic materials in each soil and how this would affect the results. The sponsors acknowledged that they did not measure total organics in soils in the *in vivo* studies, but this variable is important to investigate.

A reviewer asked whether there are *in vivo* data for other species. The sponsors said there are protocols for the rat, monkey, and others, but that study protocols vary widely. Seven substrates are being used for a weanling rat study, but at high doses; therefore, one sample in two species is all the data available for concordance.

The sponsors clarified that the soil was sieved to 250 microns to be representative of what children are exposed to. One sample (Aspen, which is mostly lead carbonate) was sieved to 150 microns, but they found that particle size of the lead phase, and frequency of lead species occurrence, was independent of sieve size.

Reviewers questioned why only one reference soil was used when soils in the U.S. vary greatly, suggesting that more reference soils be used to develop a distribution to cover matrix effects. The sponsors indicated that the purpose of the reference soil was to provide a control sample that is widely available so that labs could use it to validate or calibrate their performance.

DISCUSSION

The sponsor, EPA, requested that the reviewers evaluate and respond to a number of questions. These were discussed as follows:

1. Based on the materials presented, do the committee members believe that it is possible to develop a valid *in vitro* bioavailability test for lead and arsenic in soil?

2. Based on the materials presented, do the committee members observe any areas in which the test protocol could be improved?

3. Do the committee members believe that the validation efforts, as planned, provide sufficient statistical power for the intended purposes?

4. Is the test method of sufficient plausibility for the purpose of public health decision making?

5. Do the committee members have any suggestions for additional experimental work that should be included in the 1998 experimental program outlined above?

6. Do the committee members have any recommendations regarding other inorganic elements that the SBRC should include in their ongoing *in vitro* development research program?

1. Based on the materials presented, do the committee members believe that it is possible to develop a valid *in vitro* bioavailability test for lead and arsenic in soil?

The panel identified additional information that they thought was needed to develop a valid *in vitro* test for lead. They noted that for lead a number of drivers had been identified for absorption (for example pH), but that additional drivers such as the organic and clay content of the soil needs to be examined also. Data need to be developed with the goal of understanding the mechanisms that influence absorption. There is also a need to build more distributions for drivers to capture the variability in populations and soils. For example, low pH at a constant level would be the worst case scenario. In real life, variation in pH is likely to be represented by a distribution. More soil samples with *in vivo* data are needed to cover a broader range of soils.

Good correlations exist for the stomach between the *in vivo* and *in vitro* results; however, lead is absorbed in the small intestine. The sponsors acknowledged that the chemistry of the small intestine is very complex and the correlations are not as good.

The panel was divided over whether a valid *in vitro* bioavailability test can be developed for lead in soil. Most thought it could be done while a few disagreed. One reviewer thought it possible to develop an assay for bioaccessibility, but not bioavailability, because active transport is lacking in the *in vitro* test. In addition, the swine model was not done under fasting conditions (i.e. the soil was administered in a dough ball). Therefore, it appears as if a nonfasting *in vivo* model was compared to a fasting *in vitro* model. The sponsors indicated that the influence of the delivery system was tested. The soil included in the dough ball in the *in vivo* test was compared to the results from gavage and there were minimal differences.

For arsenic, the reviewers thought that a better understanding of the *in vivo* arsenic data are needed before moving forward. The drivers for measuring arsenic bioaccessibility are not yet known and the intestinal component may be more important than it was for lead. The reviewers encouraged the sponsors to continue their opportunistic work on arsenic while moving forward in a methodical fashion on lead.

The reviewers thought that an overall plan is needed for where the sponsors want to go with this research. The sponsors need to put together a framework describing how these data are to be used in risk assessment and management. The quality of data required will differ with different uses and exposure situations. The materials that were provided for this review reflected a number of different views on uses of this information. It is hard to respond to question number one without a clear idea of how the results are to be used. For example, results may be used for screening at a contaminated site or for development of national legislation.

2. Based on the materials presented, do the committee members observe any areas in which the test protocol could be improved?

Individual reviewers had a number of suggestions for improvements and questions on the protocols. One reviewer indicated that the current *in vitro* method is too complex if the goal is for any laboratory to be able to perform this test. The sponsors confirmed that their intent is for any lab to be able to perform the test but they think that right now it is no more complicated than TCLP. A reviewer asked whether the Maryland bioavailability method (a simple extraction method using a pH of 1.5 at room temperature overnight) would get the same results. The sponsors replied that both temperature and movement made significant differences in this test's results

To assure that the labs will do what is needed, a reviewer suggested that the Quality Assurance Project Plan (QAPP) include more details. For example, more data are needed on exactly how to make the samples, clean the glassware, and calibrate the instruments. For samples that do not meet the criteria, the QAPP should be more specific about retesting and sample acceptability. The EPA method cited (SWA846) should be attached.

Several reviewers suggested including samples of known lead concentration to help confirm that all labs are conducting the test properly. Another reviewer suggested tightening the control limits, for example the matrix spike at 75-125% is too wide. In addition, duplicate recovery at $\pm$ 20% is too much. The sponsors indicated that these values came from EPA's functional guidelines for total metals analysis for the Contract Lab Program and if they tighten the limits they would be accepting less error than EPA does for regulations. Several reviewers suggested that they should let the data dictate the control limits.

One reviewer thought that the predictivity of the test is dependent on the range of soils covered with all their variability as to particle size, moisture, and other factors. Reliance on correlations will depend on the extent of characterization of test soils and whether the "new" soil has a sufficient number of these characterizations.

More *in vivo* studies are needed with more species to build better correlations. One reviewer suggested the correlations should be stratified by species to determine which species has the best correlation with the *in vitro* model. Another suggestion was that development of a scaled down *in vivo* model might be used to build better correlations.

Another reviewer suggested that the amount of soil to solution should equate with what is in a child's stomach. One could then compare Ellen O'Flaherty's lead model for the child to the estimates of bioaccessibility of lead from the *in vitro* model.

Particle size was discussed, specifically the choice of sieving to 250 microns versus 150 microns. It was suggested that the Consortium consider *in vitro* work that determines solubility as a function of particle size. In addition, several reviewers raised the issue of pinocytosis and what size particles will be directly absorbed.

One reviewer questioned the effect on the correlations of allowing samples to sit at room temperature during extraction and filtration. The sponsors indicated that in tests they found that the sample will continue to dissolve while sitting, but they did not see a difference for up to four hours at room temperature.

A reviewer pointed out that the equation ($y = mx + b$), where "y" is bioaccessibility and "mx" is bioavailability, appears to have "x" and "y" reversed. The sponsors agreed that this was indeed wrong and would be corrected.

One reviewer asked whether the stomach secretes more acid to respond to what is added to the stomach. Does pH stay constant or does it vary, and if it varies, a varying pH should be used in the *in vitro* test. The sponsors indicated that their aim is to build an *in vitro* model to predict what is going on in the animal. If the animal is adjusting to the pH increase with the secretion of additional acid, then the model should show some buffering capacity to maintain the pH similar to the *in vivo* adjustment.

3. Do the committee members believe that the validation efforts, as planned, provide sufficient statistical power for the intended purposes?

Statistical precision, rather than statistical power, is the important issue according to one reviewer. Power is not the central concern; one should focus on getting the slope of the regression line near one and the y intercept near zero. Forcing the line through the origin will not change the slope much and is appropriate in this case since the intercept is not statistically different than zero.

One reviewer questioned how a correlation could be drawn when relative bioavailability was defined differently for the *in vivo* test than bioaccessibility was defined for the *in vitro* test. Relative bioavailability was defined as the ratio of doses, while relative bioaccessibility was defined as the ratio of solubilities, which is a ratio of the material available for absorption. The sponsors agreed to clarify this issue further.

When asked to comment on whether the number of labs in the interlaboratory study is adequate, one reviewer recommended that in general one should put resources where the variance is expected; if variance is among labs, then sample more labs; if variance is among samples, then use more samples. Several other reviewers indicated that lab results do frequently vary, indicating that the number of labs is important. Another reviewer indicated that one of EPA's research laboratories has done round robin testing of labs which might provide useful information to help answer this question.

4. Is the test method of sufficient plausibility for the purpose of public health decision making?

The reviewers all agreed that the answer to this question depends on the decision at hand. For screening level assessments for lead, the panel thought the method could be used within the limits of the characterizations of the validation efforts. For larger projects and those with high impact, however, additional work is needed. The existing method is not yet ready for arsenic.

The panel all agreed that the Consortium is on the right track and that the work is very important and useful to risk assessment. The panel encouraged additional funding to expand the already good work, especially with the large number of variables that need to be controlled.

5. Do the committee members have any suggestions for additional experimental work that should be included in the 1998 experimental program outlined above?

The panel had the following suggestions for additional experimental work:

* Find information on where different species of arsenic and other metals are absorbed; perhaps these data are in the literature or could be tested in a small non-rodent animal such as swine.

* Explore the question of whether *in vitro* and *in vivo* correlations remain the same with different particle sizes.

* With the large number of types of soils in the U.S., consider correlations between the most important components for 10-20 of the most common types of soils. Consider *in vitro* tests first and if these are predictive, then model *in vivo*.

* For arsenic rodents would not be a good model for humans.

* In addition to Superfund site soils, it would be useful to examine household dust samples and urban residential samples.

6. Do the committee members have any recommendations regarding other inorganic elements that the SBRC should include in their ongoing *in vitro* development research program?

The following points were made regarding the discussion of inorganics:

* Manganese has been a driving chemical for a number of recent risk assessments. It raises issues of nutritional requirements, with solubility and absorption the key issues, not toxicity.

* Mercury found in mining sites might involve an issue of bioavailability. While good *in vivo* studies have not been done for mercury, several papers were published recently in *Environmental Health Perspectives* on mammalian absorption.

* Cadmium might be useful to study, particularly how it is absorbed in plants. Earlier *ITER* peer review meetings (December 1996 and March 1997) have discussed cadmium and the summaries of these meetings may have some useful information.

* Chromium would not be a good candidate for soil bioaccessibility studies because hexavalent chromium is less soluble in acid conditions, as compared to basic conditions, and is reduced to trivalent chromium. Trivalent chromium would also be a poor candidate because the RfD is based on a rat feeding study involving insoluble chromic oxide; a form of chromium with limited gastric solubility.

* When considering minerals like chromium and manganese which can exist as cations and anions, the cation forms should be used rather than the anions or mixtures of the anion and cation. The bioaccessibility of the anions are very likely different from those for the cations. This may be part of the problem with arsenic.

* For copper and zinc, increased organic content of soils leads to issue of bioavailability.

* Beryllium might be useful and the latest EPA IRIS (Integrated Risk Information System) information should be viewed.

The sponsors also asked for the panel's thoughts on the relevance of pursuing indirect exposures from dust, vegetation, garden produce, and forage. One reviewer shared his recent experience of using EPA's sludge methodology to calculate exposures from

vegetables. He calculated that 95% of the dose for all the metals was from this pathway, which did not seem credible and raised questions of which metals are taken up by which parts of the plants, and when ingested how much of the metal is available? Another reviewer confirmed that knowledge of the form of the metal in the plant is very important. One reviewer pointed out that nutritionists could help with bioavailability of metals in plants, particularly for zinc and copper. It was suggested that the sponsors contact Glenn Rice of EPA's National Center for Environmental Assessment in Cincinnati as a possible source for information on how bioavailability in plants is handled in EPA's indirect exposure methods.

RECOMMENDATIONS

See Discussion above.

Managing Potential Conflicts of Interest

ITER Peer Review Meeting

(Approved by panel on April 27, 1998)

TERA peer reviewers donate their time and talents to this effort. They are selected based upon their expertise and qualifications and are employed by many types of organizations. *TERA* strives to create a balance of expertise and affiliations for each meeting. However, individual peer reviewers are representing their own expertise and views, not those of their employer.

TERA has requested that each peer reviewer identify potential conflicts of interest related to the review of the health risk assessment of butyl benzyl phthalate and the bioavailability research program and their sponsors. Each reviewer has signed a statement indicating that he or she does not have a conflict of interest concerning these chemicals. *TERA* has discussed any issues concerning conflicts or the potential or appearance of a conflict, with the reviewers, and if necessary with the *TERA* Board of Trustees. *TERA*'s recommendations are listed below. These statements were discussed at the April 27, 1998 meeting and the panel agreed to the text below. Because of the diverse nature of the two reviews and need for specialized expertise, several reviewers were selected to participate in just one part or the other of the meeting.

Mohamed S. Abdel-Rahman – Dr. Abdel-Rahman is on the faculty of the New Jersey Medical School, Department of Pharmacology and Physiology. He does not have any conflicts and will participate fully in both discussions and consensus.

John P. Christopher - Dr. Christopher works for the California Environmental Protection Agency (Cal EPA). Cal EPA regulates various aspects of production, use, sale or disposal of virtually all chemicals; but he is not currently involved in any assignment or

controversy regarding these reviews. Dr. Christopher will participate fully in both discussions and consensus.

George P. Daston – Dr. Daston works for the Procter and Gamble Company. Dr. Daston has been asked to participate as an *ad hoc* reviewer of the butyl benzyl phthalate assessment because of his expertise in reproductive toxicology. He does not have any conflicts and will participate fully in the discussions and consensus on butyl benzyl phthalate.

Joyce M. Donohue – Dr. Donohue works for the U.S. EPA, Office of Water. While Dr. Donohue works for EPA, which is sponsoring the bioavailability review, she has not been involved in this project in any way. She does not have any conflicts and will participate fully in both discussions and consensus.

Michael L. Dourson - Dr. Dourson works for Toxicology Excellence for Risk Assessment (*TERA*). He does not have any conflicts and will participate fully in both discussions and consensus.

Fran V. Kremer – Dr. Kremer works for the U.S. EPA, Office of Research and Development and has been asked to participate as an *ad hoc* review for the bioavailability review because of her experience in the area. While Dr. Kremer works for EPA, which is sponsoring the bioavailability review, she has not participated in this project. She does not have any conflicts and will participate fully in the discussion and consensus on butyl benzyl phthalate.

M.E. (Bette) Meek – Ms. Meek works for Health Canada. Health Canada prepared and is sponsoring the butyl benzyl phthalate review; therefore, Ms. Meek will not participate as a reviewer in that discussion. She has no conflicts with the bioavailability review and will participate fully in that discussion and consensus.

Rashmi S. Nair – Dr. Nair works for Solutia. She has been asked to participate as an *ad hoc* reviewer for the bioavailability review because of her experience in evaluating the toxicity of metals in soils and her general risk assessment experience. She will not participate in the butyl benzyl phthalate review. She has no conflicts with the bioavailability review and should participate fully in those discussions and consensus.

Deborah Proctor – Ms. Proctor works for the ChemRisk Division of McLaren/Hart. She has been asked to participate as an *ad hoc* reviewer because of her experience in evaluating the toxicity of metals in soils and her general risk assessment experience. ChemRisk uses similar gastric extraction studies in their work, but this does not create a conflict and she will participate fully in both discussions and consensus.

Sandy M. Roda – Ms. Roda works for the University of Cincinnati, Department of Environmental Health. She has been asked to participate as an *ad hoc* reviewer for the bioavailability review because of her experience in analytical chemistry. She does not

have any conflicts and will participate fully in discussion and consensus on butyl benzyl phthalate

Ruthann Rudel - Ms. Rudel is employed by the Silent Spring Institute. She does not have any conflicts and could participate fully in the discussions and consensus, however, she was not able to attend the meeting. She provided written comments on the butyl benzyl phthalate assessment that were discussed by the panel.

Alan H. Stern - Dr. Stern is employed by the State of New Jersey, Department of Environmental Protection. A member of his staff, Dr. Gloria Post is a representative on the Bioavailability Consortium, although the state does not contribute money to the consortium or fund any of its related work. The Department is involved in order to address and be informed on issues and aspects of the methods development on which it is likely to be asked to consider in the future. Dr. Stern has not personally been involved in consortium meetings and has been kept informed in a summary fashion by Dr. Post. Dr. Stern pointed out that the state of New Jersey provides no financial assistance to the consortium. The panel discussed this and agreed that Dr. Stern could participate fully in the discussions and polling for consensus. He has no conflicts with the butyl benzyl phthalate assessment and will participate fully in discussions and consensus for it as well.

Paul A. Succop – Dr. Succop is on the faculty of the University of Cincinnati. He has been asked to participate as an *ad hoc* reviewer for the bioavailability review because of his experience with statistical methods and analysis. He does not have any conflicts and will participate fully in the discussions and consensus for the bioavailability review.