ADDITIONAL COMMENTS ON THE DRAFT RISK ASSESSMENT FOR LIBBY AMPHIBOLE WITH EMPHASIS ON RE-ANALYSES OF THE RESTRICTED ROHS COHORT FOR DERIVATION OF A REFERENCE CONCENTRATION

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These comments constitute an addendum to the comments I posted on the Science Advisory Board (SAB) website in March, 2012, and are based on extensive re-analyses of the sub-cohort used by the U.S. Environmental Protection Agency (the Agency) for the estimation of an RfC for Libby amphibole. The focus of these comments is a discussion of my re-analyses of this dataset. In addition, I respond in these comments to some of the recommendations made by the SAB to Administrator Jackson in a draft letter dated April 4, 2012.

I have done extensive re-analyses of the dataset used by the Agency for the estimation of an RfC for Libby amphibole. This dataset is a subset of the data analyzed by Rohs et al. (2008) and includes 118 workers with 12 cases of pleural plaque. These re-analyses show that the dataset is far too small for reliable estimation of an RfC . I believe also that the Agency used an inappropriate model, the Michaelis-Menten model, for estimation of the RfC.

The Michaelis-Menten Model

This model has been widely used to study receptor binding and enzyme kinetics. In its original form, used for the analyses of enzyme kinetics, the model has only two parameters. The model has been extended by the Agency to include a third parameter. In the Agency formulation, the three parameters that can be estimated from the data are a background, a plateau, and a parameter, which I will call the 'slope'.

- The background parameter is an estimate of the fraction of the general (unexposed) population that has pleural plaques.
- The plateau estimates the fraction of 'susceptible' individuals in the population. If the plateau is below 100%, it implies that a certain fraction of the population will never develop pleural plaques no matter how large the exposure to asbestos, a dubious biological construct.
- Finally, the 'slope'[1] determines how steep the exposure-response relationship is, i.e., how quickly the exposure-response curve rises from the background to the plateau.
- **It is clear that if the plateau is equal to the background, then there is no evidence of an exposure-response relationship in the data.**

Estimating the RfC using the Michaelis-Menten and other models

I have re-analyzed the dataset provided by the Agency using both Michaelis-Menten models and logistic regression models, which are more traditional in benchmark dose analyses. For the Michaelis-Menten analyses, I used the approach described by the Agency in its draft risk

[1] The third parameter influences the speed with which the exposure-response curve approaches the plateau, but is not actually the slope in the strict mathematical sense. The slope of the exposure-response curve is not a constant and is a complicated function of all three parameters.

assessment for Libby amphibole, including the use of profile-likelihood-based lower confidence intervals for estimation of the BMCL[2]. I used the BMD software (BMDS) available from the Agency website for the logistic regression analyses. This software also uses profile likelihoods to estimate the BMCL. I confirmed that my model results were identical to those reported by the Agency for the same models. Here are my observations:

1. Although it is possible to estimate all three parameters even in this sparse dataset, the Agency fixed the background rate of pleural plaques at 1% with little justification, and estimated only the plateau and the slope. When all three parameters are estimated from the data, the estimate of background rates varies between 3 and 4.5% depending upon the lag structure chosen for the exposure[3].
2. Although I did not test directly the hypothesis of equality of background and plateau in the three-parameter models, the large standard errors I found for each of these parameters suggest that equality of these two parameters cannot be rejected indicating that these data provide little evidence of an exposure-response relationship between cumulative exposure and prevalence of pleural plaques.
3. I tried a number of two-parameter Michaelis-Menten models (background rates fixed) with different lags for exposure and with various assumptions regarding the background rates of pleural plaques. With a ten-year lag and with the assumption that the background rate is 1% (this is the Agency's chosen model), I estimated a BMCL of 0.1178, identical to the BMCL reported by the Agency. As expected, however, the estimate of BMCL depends both on the chosen lag structure and the assumed background rate. These results are shown graphically in figure 1 below.
4. In every one of the Michaelis-Menten models I used to analyze the data, the estimated standard error for the plateau is so large that the hypothesis that the plateau is equal to the background cannot be ruled out by the standard Wald test[4]. **If the Agency insists on using the Michaelis-Menten model, it is incumbent upon the Agency to show that the plateau is statistically significantly different from the background. If the hypothesis of equality of background and plateau cannot be rejected, then the Agency should recognize that the model fails to find an increase in response (pleural plaques) with increasing exposure.**
5. In addition to the Michaelis-Menten model, I have analyzed the data using logistic regression models with both cumulative exposure and average concentration[5] (cumulative exposure divided by duration of employment) as the measure of exposure.

[2] This is the lower 95% confidence limit on the benchmark dose or benchmark concentration.
[3] By the AIC criterion, the fit of the Michaelis-Menten model is worse when all three parameters are estimated in the data used by the Agency.
[4] The Agency should develop a likelihood-based test for this hypothesis.
[5] I use the terms concentration and intensity interchangeably in this document.

The concentration-response models, in particular, fit the data as well as, or better than, the Michaelis-Menten models as judged by the AIC. However, these two classes of model (Michaelis-Menten and logistic) predict very different shapes for the exposure-response curves. This finding suggests very strongly that this sparse dataset does not allow discrimination among models and is, therefore, unsuitable for the estimation of an RfC. Figure 2 shows the exposure-response relationships for some Michaelis-Menten and logistic regression models. As judged by the AICs shown in that figure, the logistic regression concentration-response models describe the data best. SAB member Dr. Sheppard suggests that a supra-linear exposure-response relationship is biologically plausible and has been observed in other contexts, such as the impact of particulate matter on cardiovascular mortality. Be that as it may, the data at issue here are too sparse to distinguish between supra-linear and sub-linear models.

6. An examination of the raw data by deciles of exposure (Table 1) also indicates that there is little evidence of a supra-linear relationship between cumulative exposure and pleural plaques. This table makes it very clear that exposure-response relationships are driven largely by the number of pleural plaques in the highest decile of cumulative exposure.
7. Because the Agency uses cumulative exposure in its analyses of the data, it divides the estimated BMCL by 60 to derive a concentration adjusted for a 70-year lifetime. The Agency then uses two safety factors of 10 each to arrive at an estimate of the RfC. In my opinion, this procedure is tantamount to using three safety factors. If the BMCL is derived for the concentration directly, then two safety factors of 10 each can be applied directly to this BMCL. For example, with lag zero, the logistic concentration-response model (see figure 1) has an AIC of 73.0 (and therefore describes the data better than the Agency preferred Michaelis-Menten model with an AIC of 74.0) with BMCL = 0.04. Using this BMCL as the point of departure and using two safety factors of 10 each yields an RfC = 0.0004, which is 20 times the RfC estimated by the Agency.

Decile	Exposure (f/cc-yr)	Cases	Subjects	Prevalence
1	0.02	1	12	0.08
2	0.04	0	12	0.00
3	0.07	1	12	0.08
4	0.09	0	12	0.00
5	0.11	0	11	0.00
6	0.14	1	12	0.08
7	0.22	2	12	0.17
8	0.32	2	12	0.17
9	0.50	1	12	0.08
10	2.29	4	11	0.36

Table 1: Rohs restricted data set divided into deciles with even numbers of exposed subjects. The second column labeled "Exposure" is the average cumulative exposure lagged 10 years in each decile. It is absolutely clear that there is no evidence of an increase in the prevalence of pleural plaques with increasing cumulative exposure except in the highest decile.

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Comments on SAB recommendations regarding the RfC

With respect to the RfC, "[t]he SAB recommends that EPA include any X-ray abnormalities (localized pleural thickening, diffuse pleural thickening, or asbestosis) as the health outcome." There are no reported cases of asbestosis in the database used by the Agency for derivation of the RfC. The definition of asbestosis requires demonstration of substantial exposure to asbestos. The SAB appears to be suggesting that all cases of interstitial fibrosis in the data be called "asbestosis" and included in the analyses. In my view this would be totally inappropriate. Pleural plaques are at least considered to be markers of asbestos exposure. In contrast, it is well known that there are many causes of interstitial fibrosis other than exposure to asbestos, a significant fraction of cases of interstitial fibrosis is idiopathic, and age is a strong risk factor for the development of this condition. Control of confounding would be particularly problematic if interstitial fibrosis were included in the analyses. Accordingly, this recommendation is inappropriate and should be withdrawn.

I take issue also with the SAB conclusion that use of the full cohort of 434 workers for confirmatory analyses is reasonable. Rohs et al. (2008) gave excellent reasons for including only a subset of 280 individuals from the original cohort of 434 workers for their analyses. I believe that, if pleural plaques are to be used for the derivation of an RfC, then the sub-cohort analyzed by Rohs et al. (2008) is the most appropriate dataset to use.

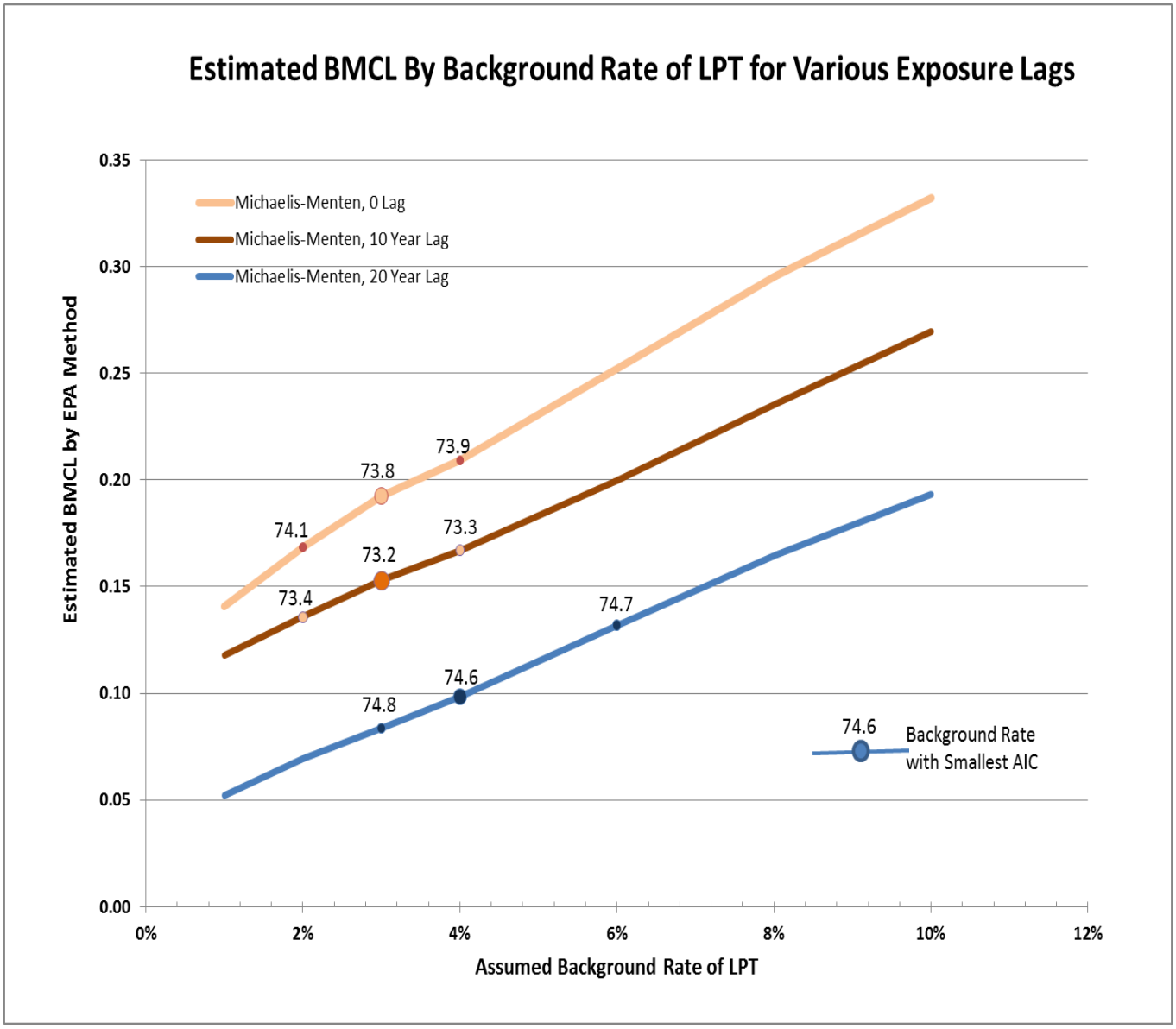

FIGURE 1: Estimated BMCLs using the Michaelis-Menten models. Estimated BMCL depends on both the lag structure for cumulative exposure and on the assumption regarding background rates of pleural plaques. For each lag (0, 10 and 20 years), the AICs are shown for various ass assumed background rates, with the lowest AIC highlighted. Note that all models describe the data about equally well.

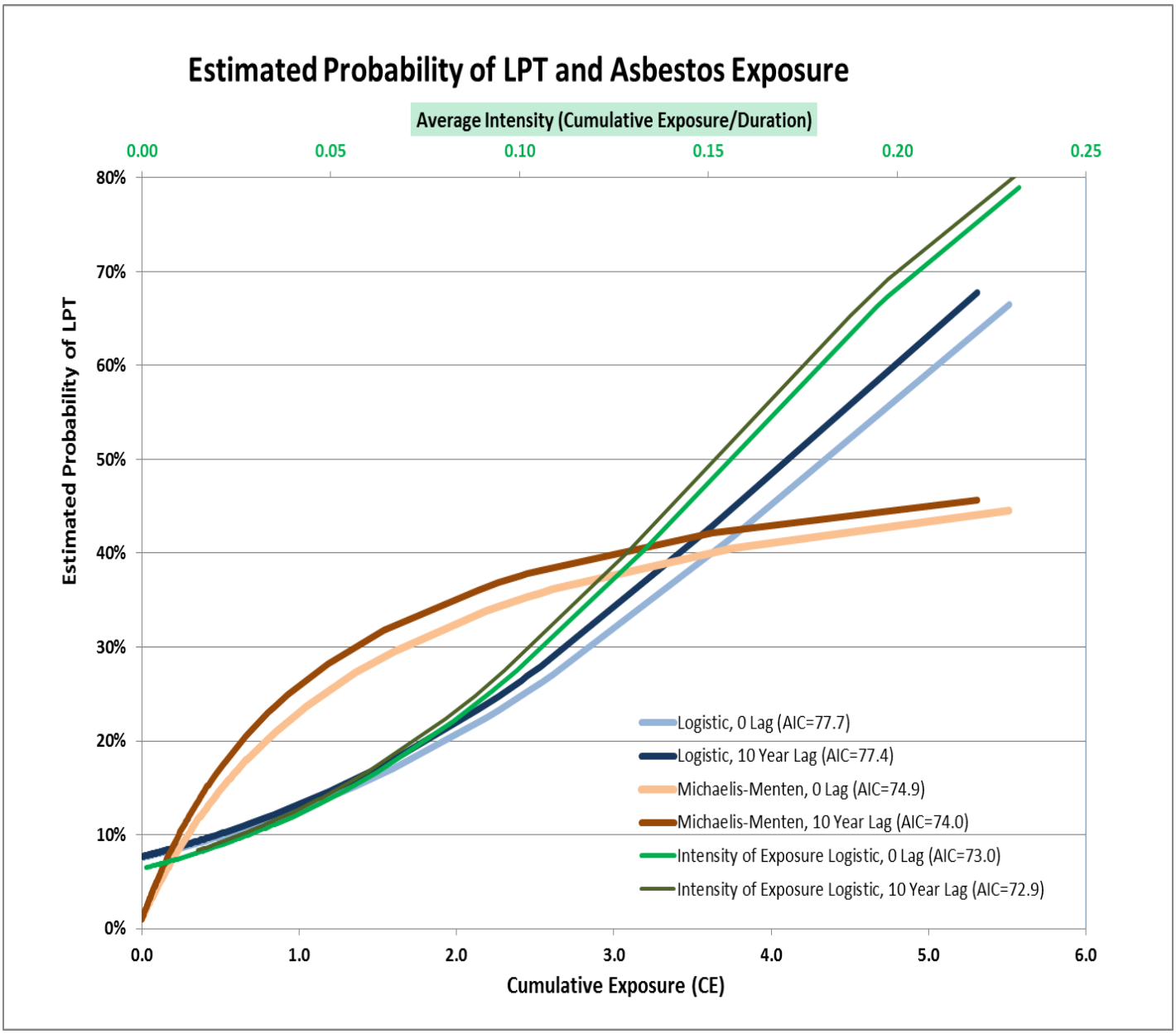

FIGURE 2: Dose-response relationships using Michaelis-Menten and logistic regression models. The two (supra-linear) curves to the left are outputs of Michaelis-Menten models with zero (AIC = 74.9) and 10 year (AIC = 74.0) lags for exposure. The four sub-linear curves to the right are outputs of logistic regression models, two with cumulative exposure as the measure of exposure, and two with average concentration (intensity) as the measure of exposure. Note that by the AIC, the sub-linear concentration-response models describe the as well as, or better than, the EPA chosen model (Michaelis-Menten with 10 year lag). Note also that when all three parameters for the Michaelis-Menten model are estimated from the data, the fit as judged by the AIC becomes worse. Therefore, the logistic concentration-response models are clearly superior.

Recommendations to the Agency

1. Much better justification is required before pleural plaques can be used as an end-point for derivation of an RfC. The inclusion of all X-ray abnormalities as an end-point makes little biological sense because the potential confounders for pleural plaques are different from those for interstitial fibrosis.
2. The dataset used by the Agency for the estimation of an RfC is too small to distinguish among models with very different exposure-response relationships. If the Agency insists on using pleural plaques for the derivation of an RfC, then a more appropriate and larger dataset should be used. The data used by Rohs et al. (2008) is a possible candidate.
3. The use of the Michaelis-Menten model needs to be better justified. What is the interpretation of the plateau? Why should a fraction of the population be immune to the effects of exposure?
4. The Michaelis-Menten model is a three-parameter model. In the absence of reliable information on the background rate of pleural plaques, all three parameters should be estimated from the data. The Agency needs to provide the appropriate analyses to show that in their preferred Michaelis-Menten model, the plateau is statistically significantly different from the background.
5. I endorse the recommendation made by the SAB Panel that the Agency analyses used for the derivation of the Inhalation Unit Risk (IUR) for cancer be extended by using models based on ideas of multistage carcinogenesis. I recommend that these extended analyses be done for both lung cancer and mesothelioma. These analyses will allow the exploration of the temporal aspects of risk following exposure to Libby amphibole. In addition to the analyses based on multistage carcinogenesis, I recommend also that the temporal aspects of risk in lung cancer be explored using conventional statistical approaches, such as the Cox model with flexible spline smoothers to investigate effect modification by age.
6. I do not agree with the SAB Panel that the Agency has chosen the appropriate dataset for the analyses. In fact, the dataset was expressly chosen to eliminate effect modification by age. Therefore, I believe that the entire Libby cohort with follow-up through 2006 should be used for estimation of the IUR. Uncertainties in exposure estimates should be addressed via monte-carlo simulations.
7. The SAB Panel appears to recommend that the algorithms used by Richardson (2008) and Zeka et al. (2011) be used to fit the data using the two-stage clonal expansion (TSCE) model. I would like to inform the Agency that better algorithms and software than those used in these publications have been developed. I would recommend using

the latest software tools[6]. I would be happy to make this software available to the Agency.

[6] See, for example, Crump KS, Subramaniam RP, Landigham CB. A numerical solution to the nonhomogeneous two-stage MVK model of cancer. Risk Analysis 2005; 25:921-926; Little MP. Cancer models, genomic instability and somatic cellular Darwinian evolution. Biology Direct, 2010; 5:19.