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MtBE and cancer in animals: Statistical issues with Poly-3 survival adjustments for lifetime studies

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Dear Sir

Goodman, et al. (2007) implemented the poly-3 survival adjustment based on maximum lifetime, discounting significant tumor findings in Sprague-Dawley rats exposed to the oxygenated gasoline additive, methyl-tertiary-butyl ether (MtBE) (Belpoggi et al., 1995, 1997, 1998). The poly-3 survival adjustment (Bailer and Portier, 1988; Portier and Bailer, 1989) was empirically derived from spontaneous tumor data using a series of 2-year studies in F344 rats and B6C3F1 mice (Portier, Hedges, Hoel, 1986), adhering to the National Toxicology Program's carcinogenesis bioassay protocol (Bucher, 2002). The operating characteristics of the poly-3 adjustment beyond a two-year time frame have never been validated. Therefore, implementation of this approach to studies longer than two years must be carefully considered. Importantly, it can be shown that applying the poly-3 adjustment to longer term studies without proper regard for its assumptions and limitations may result in erroneous conclusions.

Based on a Weibull distribution for tumor onset, the poly-3 survival adjustment for animal i is

$$\frac{(t_i/T)^3}{1}$$

if animal i was free of the tumor and died before the end of the study
if animal i had the tumor or survived to the end of the study

where t_i is the time at death from study start and T is the length of the study. Survival adjustment terms are summed within each dose group to give the effective number of animals at risk. While Portier et al. (1986) showed that tumor onset could be modeled well by the Weibull distribution up to 118 weeks of age, there is no evidence that tumors occurring beyond 2 years on study follow this distribution. In lifetime studies that exceed two years, such as the MtBE study and others conducted by the Ramazzini Foundation (Soffritti et al., 2002), using T =maximum lifetime rather than two years dramatically reduces the contribution of tumor-free animals to the effective number of animals at risk. If the control group, in particular, contains more tumor-free animals than do the treated groups, this may lead to failure to detect a significant increase in tumors, as we show below. Furthermore, when T =maximum lifetime, the poly-3 weights

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and the resulting analyses are highly influenced by the longest living animal; this animal may have lived substantially longer than all of the others in the study.

The authors used $T=174$ weeks – much longer than 2 years ($T=104$ weeks) – in three separate cases, dealing with the timing of when Leydig cell tumors (LCTs) occurred in the test animals. Using the same three cases, we explored using $T=104$ weeks in the poly-3 adjustment. We assigned a poly-3 survival adjustment of 1 to each animal that died free of LCTs after two years. Results for Case 1 are presented in Table 1.

In our reanalysis, both the high-exposure group comparison with the control group and the trend test were significant ($p_H=0.026$ and $p_T=0.008$, respectively). Our reanalyses of Cases 2 and 3 yielded nearly identical results (Case 2: $p_H=0.023$ and $p_T=0.007$; Case 3: $p_H=0.024$ and $p_T=0.008$). In the Table 6 analysis for Case 1 presented by Goodman et al. (2007), neither the individual pairwise p-values ($p_L=0.41$ and $p_H=0.19$) nor the test for trend ($p_T=0.17$) were significant; Cases 2 and 3 were similar.

The discrepancies between our analyses and the authors' analyses are a direct result of the poly-3 weights assigned to animals dying without an LCT. For example, in our analyses, an animal dying at 2 years without an LCT receives a weight of 1; the animal is assumed to have lived long enough to contribute fully to the evaluation. The authors would assign that same animal a weight of $(104/174)^3=0.21$; the animal contributes 1/5 as much to the risk of developing an LCT as an animal dying at 174 weeks. To illustrate the impact, in Case 1, the effective number of animals at risk in the control, 250 mg/kg, and 1000 mg/kg groups in our analyses are 37.8, 37.6, and 40.4, respectively, whereas they are 11.6, 13.1 and 22.7 in the authors' analyses. Because the tumor-free animals were assigned relatively small weights in the authors' analyses, the effective numbers of animals at risk were reduced, increasing the poly-3 LCT incidences. There were more tumor-free animals in the control group than in the 1000 mg/kg group, so the poly-3 incidence in the control group was increased proportionately more than, *and closer to*, that in the 1000 mg/kg group. Thus, the authors did not detect a significant increase or trend.

We also note that p-values in Table 6 of Goodman et al. (2007) appear to be two-sided, even though the authors stated that they were one-sided.

After contacting Dr. Belpoggi, we analyzed the data using the exact death times and the poly-3 test as outlined above, resulting in $p_L=0.39$, $p_H=0.03$ and $p_T=0.01$; these numbers do not fall within the range of p-values seen for the “extreme” cases chosen by Goodman et al. Incidentally, Portier, Hedges and Hoel (1986) evaluated the lethality and appropriate poly-k adjustment for interstitial cell tumors of the testes in Fischer 344 rats and found them to be clearly non-lethal with an estimated Weibull shape parameter of 4.1; hence a poly-4 test may be more appropriate here.

Finally, we are surprised that the authors were not able to obtain the death times from Dr. Belpoggi or from the Ramazzini Foundation web site, and we are equally surprised that the Journal of Regulatory Toxicology and Pharmacology would accept this article without the correct data included in the analysis.

Sincerely,

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References

- Bailer AJ, Portier CJ. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* 1988;44:417–431. [PubMed: 3390507]
- Belpoggi F, Soffritti M, Maltoni C. Methyl-tertiary-butyl ether (MTBE) – a gasoline additive – causes testicular and lymphohaematopoietic cancers in rats. *Toxicol Ind Health* 1995;11:199–149. [PubMed: 7491635]
- Belpoggi F, Soffritti M, Filippini F, Maltoni C. Results of long-term experimental studies on the carcinogenicity of methyl tert-butyl ether. *Ann N Y Acad Sci* 1997;837:77–95. [PubMed: 9472331]
- Belpoggi F, Soffritti M, Maltoni C. Pathological characterization of testicular tumours and lymphomas-leukaemias, and of their precursors observed in Sprague-Dawley rats exposed to methyl-tertiary-butyl-ether (MTBE). *Eur J Oncol* 1998;3:201–206.
- Bucher JR. The National Toxicology Program rodent bioassay: designs, interpretations and scientific contributions. *Ann N Y Acad Sci* 2002;982:198–207. [PubMed: 12562638]
- Goodman JE, Gaylor D, Beyer LA, Rhomberg LR, Beck BD. Effects of MTBE on Leydig cell tumors in Sprague-Dawley rats: Range of possible poly-3 results. *Regul Toxicol Pharmacol*. 10.1016/j.yrtph.2007.05.002accepted 2007
- Portier CJ, Hedges JC, Hoel DG. Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res* 1986;46:4372–4378. [PubMed: 3731095]
- Portier CJ, Bailer AJ. Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam Appl Toxicol* 1989;12:731–737. [PubMed: 2744275]
- Soffritti M, Belpoggi F, Minardi F, Maltoni C. Ramazzini Foundations cancer program: history and major projects, life-span carcinogenicity bioassay design, chemicals studied, and results. *Ann N Y Acad Sci* 2002;982:26–45. [PubMed: 12562627]

A Poly-3 adjusted Analysis of Leydig Cell Tumors (LCTs) With T=104 Using the Data for Case 1 from Goodman et al. (2007).

Table 1

Case 1	No. with LCTs	No. at risk ^a	Poly-3 LCT Incidence	Pairwise test ^b	Trend test ^c
Controls	3	37.8	0.08		
250 mg/kg/d	5	37.6	0.13	p _L =0.45	
1000 mg/kg/d	11	40.4	0.27	p _H =0.026	p _T =0.008

^a Each dose group started with 60 animals

^b Pairwise comparison with the control rate using Fisher's exact test

^c Cochran-Armitage trend test on poly-3 adjusted rates