LINE BY LINE COMMENTS OF THE NORTH AMERICAN POLYELECTROLYTE PRODUCERES ASSOCIATION

Page & Line	Comment
Page 4: Line 26	There are dose response studies from both Tyl and Chapin. The issue of no-effect doses is covered in great detail in the review by Tyl and Friedman, Reprod Toxicol 17:1-13, 2003. There are well defined no-effect levels for dominant lethal mutations in both rats from Tyl *et al* and mice Chapen *et al*. These values should be cited.
Page 5: Line 22	The concept of adducts and PBTK model needs to be revisited as there is no discussion about the relationship between adducts and response. Hemoglobin and DNA adducts track very well. It is NAPPA's opinion based on in depth evaluation and unpublished data that these are indexes of exposure and not of response. If the SAB disagrees, there should be some citation that adducts represent an indicator of response rather than exposure. That the adducts represent an index of effect should be considered speculation without documentation.
Page 6: Line 13	In the discussion of relevance to man, there is no speculation on the significance of the tumors or the dose and time-to-tumor in animals. The tumors produced by acrylamide are not human relevant tumors but rather are specific to the Fischer 344 rat (see EPL Rpt. 750-001 *Acrylamide: A two-year drinking water chronic toxicity-oncogenicity study in Fischer 344 rats a histopathology peer review and pathology working group review of proliferative lesions involving the mesothelial lining cells of the tunica vaginalis of the testes in a two-year drinking water chronic toxicity-oncogenicity study in Fischer 344 rats with acrylamide*, which has been provided to the SAB and EPL report *induction of tunica vaginalis mesotheliomas in rats by xenobiotics*. also see environ report dated Dec. 12, 1999, *mechanism of acrylamide induction of benign mammary fibroadenomas in the aging Fischer 344 rat: relevance to human health risk assessment*.
Page 7: Line 19	The Sumner study is the only one with inhalation exposure and metabolism and yet there is no discussion of this study. Whatever model is selected for PBTK modeling needs to be validated against Sumner *et al*, "*Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure*." Toxicol Sci 75:260-270, 2003. This is the best inhalation study available. They did not observe 100% absorption in that nose-only inhalation study in rats and mice. This is a highly critical issue because the inhalation value represents the primary impact of the EPA assessment.

Page 9: Line 25	The lesions measured were very sensitive indexes. The measurements in Johnson et al were highly sophisticated using imaging. These were not toxicologically significant lesions but served as an early indicator of possible effects. For the purposes of establishing uncertainty factors, it must be made clear whether these are nuerotoxic lesions or preneurotoxic lesions. Our cage side observation of the animals and the pathologist's evaluation of the lesions suggested that these were preneurotoxic lesions. This issue must be resolved.
Page 11: Line 13	There is no recognition of the clinical progression of the disease. Acrylamide neuropathies are well recognized and documented. Extrapolation from animals is unnecessary. With regard to discussion on the mode of action of acrylamide, there is little discussion of the clinical aspects of the neuropathy, which have been well documented. These should be elaborated. There were no clinicians on the SAB with experience with acrylamide neuropathies.
Page 13: Line 14	Not all of the dominant lethal studies were conducted at 50 mg/kg. Tyl had an excellent dose response Dose response of acrylamide in the dominant lethal test is presented in Tyl et al (2000). "*Rat two-Generation Reproduction Study and Dominant Lethal Study of Acrylamide in Drinking Water*." Reproductive Toxicology 14:385-401, 2000**.** The NOAEL is 5 mg/kg-d when administered for 13 weeks. The reproductive deficits were completely accounted for by these dominant lethal mutations (see Tyl and Friedman *"Effects of acrylamide on rodent reproductive performance*." Reprod Toxicol **17**(1): 1-13, 2003).
Page 13: Line 17	The Russell study was positive was positive for specific locus mutations.
Page 14: Line 14	Crofton evaluated dose response versus qualitative response. Crofton et al, "*The impact of Dose Rate on the Neurotoxicity of Acrylamide: The Interaction of Administered Dose, Target Tissue Concentrations, Tissue Damage, and Functional Effects*." Toxicology and Applied Pharmacology **139**: 163-176, 1996. contains a comparison of various dose regiments, dose duration, and microscopic and behavioral toxicity. Behavior appears to be an insensitive indicator of acrylamide neurotoxicity. Based on these studies, behavior appears to be an insensitive indicator of neurotoxicity. Similarly, in humans, clinical observation of acrylamide poisoned individuals reveals that tactile sensation is the most sensitive clinical endpoint. Once tactile sensation is validated, it can be confirmed by EMG. Virtually all medical personnel familiar with acrylamide know this.

Page 15: Line 9	Hagmar had problems concerning when individuals were exposed and the measurements of signs of neurotoxicity. The only relevant study is Calleman/Costas, which was a sound clinical study. We agree that the Hagmar study did not have sufficient data for establishing an RfD and RfC. That is not the case for Calleman and Costas, though. Hagmar had issues with duration of exposure and time subsequent to cessation of exposure that were not shared by Calleman and Costas.
Page 15: Line 22	There was no NMA in the Costas study. Furthermore including a population with exposure to other neurotoxicants would only increase the conservatism of the evaluation.
Page 16	The discussion of BMD is interesting and application of BMD to tumor formation is a matter of policy. Nevertheless SAB excludes BMD for genotoxicity and uses point estimates. The SAB should recommend that EPA use slope estimates generated through curve fitting with EPA's BMD methodology rather than point estimates. The Allen et al approach should be encouraged by SAB as sound science.
Page 17: Line 20	References are needed for the rat being less sensitive to neurotoxicity. In the case of acrylamide, rats are more sensitive than mice. Nevertheless, it is surprising that no recommendation is put forth to study rats. McCollister found dogs to be less sensitive than rats, also. That the rat is less sensitive than other species needs a reference. McCollister has shown the opposite. This statement needs to be revisited. Cats appear to be uniquely sensitive to acrylamide.
Page 17: Line 20	SAB concludes that the tumors are of genotoxic origin since all other agents causing these 2 tumors in rats are genotoxic. There are only 2 chemicals that cause both tumors. One is a structural congener of acrylamide (glycidol) and the other is ethylene oxide (not statistically significant). Relevance of the TVMs vis-à-vis humans has been the subject of a PWG the report of which was sent to SAB. The SAB should rethink this line of reasoning as it is only based on 2 chemicals. The SAB is critical of the non-genotoxic mechanisms because they have not been established when this conclusion is based on 2 chemicals.
Page 32: Line 26	The Dearfield assumptions are not transparent. He assumes 10,000 more potentially sensitive sites on the human genome than the mouse. Mice and humans share the same number of genes so the derivation of this number is questionable. There is no correction for PBTK. The Allen approach is consistent with the EPA Guidelines and fully transparent. The SAB should recommend using the Allen methodology, which represents curve fitting rather than the parallelogram approach of Dearfield. The Dearfield approach uses one experimental point rather than the slope of the dose response curve. This parallelogram methodology is now obsolete.

Page 33: Line 2	The parallelogram and the DD are point estimates and inconsistent with EPA guidelines. This methodology is not clear. There is a factor of 10,000 used in this methodology, which has not been elucidated. The SAB should review the original publications on this methodology.
Page 33: Line 4	These low dose values may also enormously overestimate risk due to alternative modes of action, repair and overestimation of the conversion from the mouse to human genome. Even with these assumptions, solving the Dearfield equation for environmental and workplace exposure results in an effect of 1.9 genetic diseases per 10^{-6} offspring. From a molecular biology/mutagenesis point of view, it is more likely that these methods overestimate risk. This whole line of reasoning should be eliminated. The BMD is a valid/transparent expression of risk.
Page 35: Line 11	To say there is a “paucity of new data” since the Dearfield paper is erroneous. There are an enormous number of acrylamide publications since 1995 when the Dearfield paper was published. There is an interesting publication modeling the cumulative response in the micronucleus showing no cumulative risk from acrylamide. This is covered in the Allen review.
Page 38: Line 7	Reclassification of tumors in a published, GLP study, which were read by a board certified veterinary pathologist, without even reviewing the slides or contacting the pathologist is incomprehensible. These tumors were morphologically distinguishable from the gliomas. Surprisingly, the SAB did not comment on the precancerous glial proliferations, which were called tumors, which today would likely not be called tumors. This whole issue of the response of the brain should not be in the SAB report for the reasons cited here. If the SAB has questions about the response, the scientific way to go about clarifying them would be to recommend a PWG for the brains, not to draw a conclusion based on no data.
Page 38: Line 13	“All rat brain tumors are caused by DNA reactive chemicals or radiation.” is not true. Close review of Sills *et al*, "*Examination of low-incidence brain tumor responses in F344 rats following chemical exposures in National Toxicology Program carcinogenicity studies." Toxicol Pathol* 27(5): 589-99, 1999 reveals that there are many substances that cause primary brain tumors. Parenthetically we suspect that the SAB is referring to gliomas and not neuromas.
Page 38: Line 19	There are only 2 substances which causes statistically significant increases in gliomas and peritoneal mesotheliomas; glycidol, at 75 mg/kg (acrylamide was doses at 2 mg/kg) and ethylene oxide. Close review of the literature does not support this general allegation. A detailed report on this issue is being prepared by EPL under contract with NAPPA. This report is expected in July.

Page 38: Line 25	The mouse tumor initiation promotion study has not been considered a carcinogenicity study for a long time. It is interesting and perhaps may have some relevance but the mechanism for this induction is not active in humans. When a chronic mouse study is completed, then mouse can be considered. We are providing a list of references, which demonstrate that the mouse tumor/initiation studies are not relevant to man. Furthermore, SAB should point out that acrylamide was not a complete carcinogen in this assay.
Page 39: Line 27	The importance of the study by Oleson has been exaggerated. Women with a high fried food diet as suggested by acrylamide adducts had a potentially higher incidence of postmenopausal breast cancer than the case controls. There was no correlation with glycidamide adducts. If this article is true, cancer correlates with acrylamide and not glycidamide. Since acrylamide is not mutagenic, unlike glycidamide, the tumors would have to have been caused by a non-genotoxic mechanism. These are hormonally sensitive tumors so there is some biological plausibility. However, this would require an entire rethinking of the risk assessment and the PBT model.
Page 40: Line 14	Only glycidol and ethylene oxide produce these tumors. As we stated earlier, induction of these tumors is not a universal characteristic of genotoxic substances. Only these 2 substances do it. The mesotheliomas are morphologically, and pathologically substantially different from those induced by both EO and acrylamide and cannot be compared. They were metastatic and the cause of death in the rats on test.
Page 40: Line 16	This is circular reasoning. There was no increase in brain tumors so how can this be evidence for something that did not take place. Induction of brain tumors by acrylamide is highly suspect. To conclude that these suspicions are proof of acrylamide being a genotoxic carcinogen is illogical.
Page 40: Line 20	These studies were conducted in young rats. Regulation of the thyroid changes as rats changes with age. The conclusion of this study is that acrylamide had no effect on the thyroid gland. Yet there are many references in the literature to hormonal effects under these conditions. There is an inconsistency here. The Bowyer study found no changes in gene expression in the thyroid gland and the pituitary and hypothalamus. Yet the authors concluded that this is evidence for acrylamide genotoxicity. Certainly if acrylamide were genotoxic, changes in gene expression would have been observed in this study. Adducts are formed all through the body so the pituitary and hypothalamus would also have been effected.
Page 41: Line 9	This sentence is backwards. The genotoxicity is shown at high doses. Cell proliferation occurs at low doses. Acrylamide is a very weak mutagen. Allen calculated its BMD10 at ~40 mg/kg-d. The induction of cell proliferation and hormonal effects appear to occur at lower doses. This is a point the SAB has continued to miss.

Page 43: Line 2	This objection is not clear. The protocol for this study was conventional to investigate the high dose and control and any animals dying on test. With no effect in the high dose, it is unnecessary to look at a lower dose since we already know the higher dose is clean. If an effect were observed at the low dose and not the high dose it would have to be ignored. An objection is raised about the percentage of spinal cords lost in this study. There is no reason to believe that there was a bias in spinal cords so the tumor incidence in the remaining spinal cords should have been the same as in those which were lost. A sufficient number of spinal cords were studied to draw a conclusion.
Page 43: Line 5	This sentence is reflective of the problem with the SAB not having a pathologist available and not reading the report of Dr. McConnell who wrote the criteria for classifying tumors for the NTP/NIEHS. Malignant reticulosis is a lymphatic tumor and cannot be combined with gliomas, which are neural tumors. These tumors were morphologically distinguishable from gliomas and were reported as such. That there incidence was unusual was the reason we reported the finding even though they were not statistically significant.
Page 43: Line 26	There is no documentation of the audit of Tegeris Laboratory conduct of this study. Rather there is a memo of a meeting to discuss the audit. Industry was never contacted and when we found out, a response was prepared to the meeting minutes and sent to FDA. FDA must be asked to provide the audit report, as industry has been unable to obtain it under FOIA. A detailed and comprehensive report was submitted to FDA answering all of their alleged findings in the Tegeris study. They have not provided us a copy of the audit report under FOIA or to Tegeris laboratories during the study. They elected not to notify the sponsors during the study, either.
Page 44: Line 4	This is mistaken. There was no 0.5 mg/kg dose in females in the Friedman et al study. The increase referred to may be in fact at 1.0 mg/kg and is solely attributable to fibroadenomas, which are not of mammary origin. Again a problem of not having a pathologist on the panel. This needs to be corrected before we can comment
Page 44: Line 12	Tumors of glial origin were combined. Glial tumors were combined in the Friedman study. Gliomas, oligo-dendrogliomas and astrocytomas were all combined. This sentence should be deleted. The following sentences are unclear “what reference is this…”
Page 44: Line 16	The suggestion that there was an increase in female rat brain tumors is not correct. The incidences were 0, 2, and 3 and none were statistically significant. For statistical purposes the controls were combined. They were separated to determine the variability amongst the controls. Since there were no tumors in the controls, there was no variance amongst them.

Page 44: Line 17	Extent of evaluation of the oral tissue makes no sense. The suggestion that the documentation of evaluation of oral tissue was inadequate is inappropriate and wrong. Tumors of the mouth of the rat are not difficult to identify grossly, particularly in the cage-side observations. This sentence should be deleted.
Page 47: Line 16	NCTR has released information that acrylamide in contrast to glycidamide is not active in the neonatal rat study. While glycidamide was active in this test, acrylamide was not. Since acrylamide is the subject of this review, early life stage is not relevant to this risk assessment.

Relevance of Mouse Skin Studies

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