# Comments on Acrylamide-Associated Genotoxicity as Presented in the Draft IRIS Document

presented to the EPA-SAB on behalf of the North American Polyacrylamide Producers Association (NAPPA)
March 10-12, 2008

**Errol Zeiger, Ph.D., J.D.**

**Errol Zeiger Consulting**
**800 Indian Springs Road**
**Chapel Hill, NC 27514**
**919-932-3778**
**zeiger@nc.rr.com**

# brief summary

Acrylamide is a weak genotoxin in mouse somatic cells, and mouse and rat germinal cells.

Although the metabolite,glycidamide, forms DNA adducts, the WOE of the genetic toxicity data support the conclusion that the genetic effects seen can be explained by effects other than a direct genotoxic mechanism, specifically through interference with the mitotic and meiotic apparatus, and through induction of an oxidative stress response.

The in vivo responses seen are also consistent with a non-linear (threshold?) response.

## There are 4 aspects of acrylamide-induced genetic toxicity that I will address with respect to the conclusion in the draft IRIS document that data support a mutagenic MOA

1. The draft document does not given equal consideration to all relevant modes of action in addition to the genotoxic mode, as is recommended in the EPA draft *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity (Sept. 2007)*.

2. Although acrylamide can alkylate DNA via its metabolism to glycidamide, those alkylations are not translated to into mutations.

3. A number of the responses being interpreted here as evidence for gene (point) mutations can be equally well interpreted as evidence for gross chromosome damage.

4. The dismissal of the analyses presented in Allen *et al.* (2005) is misplaced and inaccurate.

## 1. The draft document does not given equal consideration to all relevant modes of action in addition to the genotoxic mode, as is recommended in the EPA draft *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* (Sept. 2007)

The EPA draft *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* [EPA 120/R-07/002-A] states, (in §2.4.3.3) among other similar statements, **"If there is evidence for more than one mode of action, each should receive a separate analysis."**

This has not been done, although there is compelling evidence to support modes of genotoxic action based on:

- oxidative stress and a secondary response to oxygen radicals, and
- interference with mitosis and meiosis, resulting in aneuploidy

## 1. The draft document does not given equal consideration to all relevant modes of action in addition to the genotoxic mode, as is recommended in the EPA draft *Framework*

*- oxidative stress and a secondary response to oxygen radicals*

Mammalian cell mutants isolated after treatment with AA or GA are primarily caused by loss of heterozygosity and tend to result from chromosome deletions (Jiang 2007, Koyama 2006, Mei 2008). These deletions have been attributed by some of the authors to be the consequence of oxidative stress, *i.e.*, increased levels of reactive oxygen species and depletion of glutathione which is responsible for detoxification of active oxygen species.

In a human lymphocyte comet assay (Thielen 2006), the induction of DNA strand breaks by GA required FPG, which recognizes 8-OHdG sites, which result from oxidative damage. The time course of these breaks paralleled the induction of *Hprt* mutants.

Two recent studies on the induction of gene expression by AA and GA (Clement 2007; Hasegawa 2008) showed that the principal increases in gene expression were in genes associated with detoxification and oxidative damage-related enzymes, including those in the glutathione and glutathione-*S*-transferase pathways.

## 1. The draft document does not given equal consideration to all relevant modes of action in addition to the genotoxic mode, as is recommended in the EPA draft *Framework*

*- interference with mitosis and meiosis, and aneuploidy*

Both AA and GA interfere with microtubule motility (and therefore chromosome migration) and disassembly (required for motility) in vitro (Sickles 1995,1996,2007).

AA also induces increases in aneuploidy and polyploidy in mouse bone marrow cells (Shirashi 1978; Gassner 1996; Schriever-Schwemmer 1997), and mitotic spindle damage in splenic cells (Backer 1989). In male mouse germ cells it induces meiotic delay, and increases in aneuploidy and hypoploidy (multiple studies).

## 2. Although acrylamide can alkylate DNA via its metabolism to glycidamide, those alkylations are not translated into mutations

*- based on gene expression studies and the kinetics of alkylation and the accompanying genetic effects.*

DNA damage/repair genes are not induced by AA or GA in human cells or *C. elegans*; the induced genes/gene families were associated with responses to oxidative stress (Clement 2007; Hasegawa 2008). *GADD45*, a first-responder to DNA damage was down-regulated.

Nearly all the AA-induced alkylations in mouse sperm are associated with the protamines (Sega 1989,1990) which would prevent normal chromatin condensation in maturing sperm, and supports the conclusion that the dominant lethal and heritable translocation events are secondary responses to the protamine, rather than the DNA, alkylation.

DNA adducts are persistent with half lives of approx. 53-89 hrs (Doerge 2005); the DNA damage measured in the comet assay appeared to peak at 2-5 hrs (Maniere 2005). This provides evidence that another mechanism, such as oxidative stress, may be operating, and that the GA-adducts were not responsible for the genetic effects.

## 3. A number of the responses being interpreted here as evidence for gene mutations can be equally well interpreted as evidence for gross chromosome damage

*- including in vitro mammalian cell mutagenicity, in vivo mutations in the BigBlue transgenic mouse, and male germ cell specific locus mutations.*

94% of the large colony mutants (considered to reflect gene mutations) and 100% of the small colony mutants (considered to reflect chromosome mutations) induced in mouse lymphoma L5178Y cells by AA and GA are deletions (Mei 2008). The majority of AA-induced mutants in human lymphoblastoid cells were deletions, although the majority of GA-induced mutants were not (Koyama 2006).

In the BigBlue assay, AA and GA are more effective in inducing *Hprt* mutants than *cII* mutants; the *Hprt* locus will detect deletions much more readily than the *cII* locus (Manjanatha 2006).

The mouse specific locus mutations that were examined were shown to result from chromosomal deletions.

## 4. The dismissal of the analyses presented in Allen et al. (2005) is misplaced and inaccurate

Many of the "serious (if not fatal) flaws and assumptions" [§4.8.3.1. *Hypothesized Mode of Action—Mutagenicity* (at pg. 151)] attributed to the Allen (2005) categorical regression analyses of in vivo genetic toxicity data, are also used by the EPA in their analyses.

*E.g.* the use of the $BMD_{10}$ calculation, extrapolating from high doses and limited sample sizes, and "disregarding the one hit, one tumor hypothesis."

Allen B, Zeiger E, Lawrence G, Friedman M, Shipp A. Dose-response modeling of in vivo genotoxicity data for use in risk assessment: some approaches illustrated by an analysis of acrylamide. *Regul Toxicol Pharmacol*, 41, 6-27, 2005.

**Allen et al. "… [assumed that] it is acceptable to apply a benchmark response of 10% [*i.e.*, a $BMD_{10}$] to mutagenic events assumed to lead to tumor formation when the generally accepted "minimal" risk level for carcinogenicity is 0.0001% …."**

Response:

§5.4 [*Cancer Assessment*] of the draft IRIS document calculates and uses $BMD_{10}$ and $BMD_{20}$ values for estimating cancer risks.

**“extrapolations from very high doses, and limited sample sizes”**

Response:

This is the traditional approach for using rodent cancer and genetic toxicity data. The EPA in its analyses uses data derived from similar short-term, high-dose rodent cancer and genetic toxicity protocols to support its conclusions.

## “disregarding the one hit, one tumor hypothesis”

Response:

Traditionally, experimental data and their analyses are used to test, and drive, the hypothesis; the hypothesis is not supposed to dictate the interpretation of the data. One of the aims of the IRIS document is to determine whether the one-hit/one-tumor, or an alternative model, is more appropriate for this chemical.