

# **An Analysis of Acrylamide Genotoxicity**

*Submitted to*

*The Committee on Mutagenicity*

*by*

*The Polyelectrolyte Producers Group*

*August 2007*

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## Summary

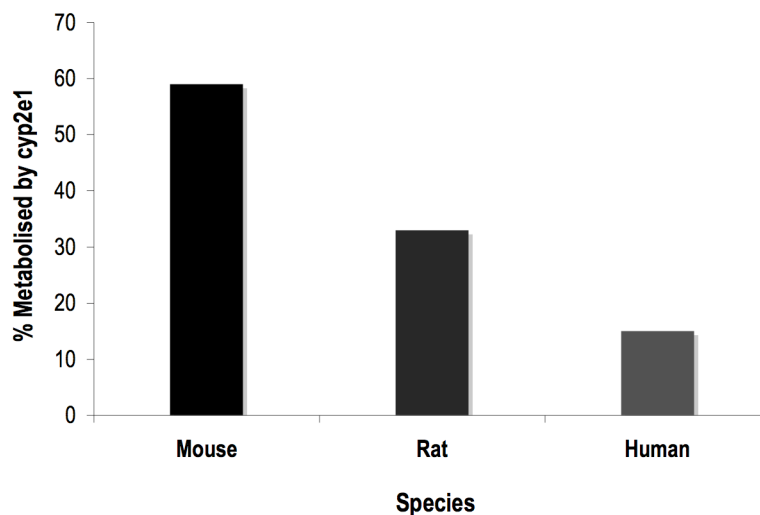
Acrylamide is a weak clastogen which acts through protein binding rather than DNA binding. Data in the *in vivo* micronucleus test show a clear NOAEL dose of 4 mg/kg/day for MN-PCEs and a clear threshold/non-linear response beginning at 2 mg/kg/day for MN-NCEs (Davis and Recio, 2007). This dose at which no genotoxicity is expected in mice – the most sensitive species – is more than 100 times higher than workplace exposure and 5,000 times higher than the daily intake through the human diet. At low environmental and workplace levels, acrylamide does not represent a human health hazard. The evidence supporting this conclusion is briefly summarized below:

1. Acrylamide adducts to haemoglobin and the 7-position in guanine.
2. DNA breaks are unrelated to sites of alkylation and are rapidly repaired.
3. Prokaryotic cells are not mutated by acrylamide.
4. Induction only of chromosome aberrations in mammalian cells in culture.
5. Acrylamide inhibits kinesin and causes oxidative stress *in vivo*.
6. The Big Blue mouse data confirm that frameshifts, rather than base-pair substitutions, are the major component of the increased mutagenic response *in vivo*.
7. The data reveal a threshold in the micronucleus test *in vivo*.
8. Acrylamide binds to protamines *in vivo*.
9. The induction of heritable translocations is non-linear.
10. Acrylamide is a very weak clastogen *in vivo*.

## 1. Acrylamide adducts to haemoglobin and the 7-position in guanine in mammalian cells

Acrylamide is metabolized via *cyp2e1* to its epoxide, glycidamide (Sumner *et al.*, 1999). The areas under the curve (AUC) for acrylamide and glycidamide are proportional to the haemoglobin adducts formed by both substances, and reflective of conversion of acrylamide to glycidamide (Fennell *et al.*, 2005). The mouse produces considerably more glycidamide than the rat, which produces more than humans (Fennell *et al.*, 2005). As shown in Figure 1, when urinary metabolites from acrylamide are compared, 59% of acrylamide goes through the glycidamide pathway in the mouse in comparison to 33% in the rat and 15% in humans (Fennell *et al.*, 2005). Similarly, N7-glycidamide-guanine DNA adducts are produced in mouse liver at more than twice the frequency that is observed in rats (Doerge *et al.*, 2005). To put this finding into context, it is important to remember that virtually all mutagenicity studies have been conducted in mice.

Figure 1 : Comparison of Mouse, Rat and Human Acrylamide Metabolism



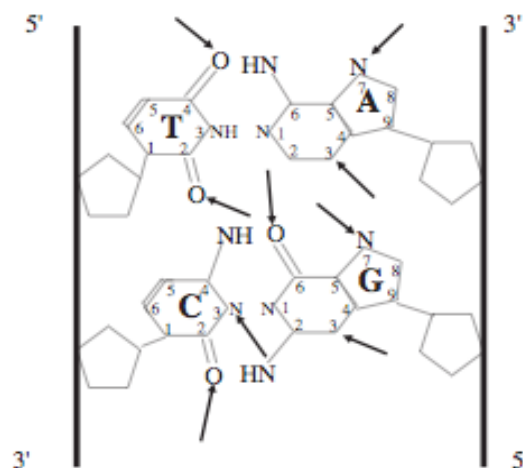
Data from Fennell *et al.*, 1995

Glycidamide is an extremely stable epoxide; so stable that it is found in mouse, rat and human urine (Fennell *et al.*, 2006). This epoxide reacts slowly with guanine to produce *N*-7-(2-carbamoyl-2-hydroxy-ethyl)guanine and, to a lesser extent, *N*-3-(2-carbamoyl-2-hydroxy-ethyl)adenine (Doerge *et al.*, 2005).

Adducts of guanine have been modeled making use of semi-empirical QM calculations to compare their stabilities (Atay *et al.*, 2005). Of the 3 potential adduction sites, namely O, N-7 and N-3, the N-7 is substantially more favorable by 8 to 10 kCal/mol. For glycidamide, the difference between the stability of the O alkyl and the N-7 adduct is 6 kCal/mol. Not only do these thermodynamic considerations explain the pattern of alkylation, they also point to the depurination reaction as the sequella of alkylation and not base pairing errors. These adducts are not in the base-pairing region (see Figure 2 below) and are not involved with induction of gene mutations (Swenberg *et al.*, 1985).

The concept of the low mutagenic potential of the N-7 adduct is supported by the data presented by Jenkins *et al.*, (2005) which indicates that N-7 alkyl adducts are effectively repaired and thus they show threshold dose response relationships for mutation induction.

**Figure 2: Double-stranded DNA showing the sites of DNA adduct formation including the important targets for alkylators (N7G, N3A, O6G, etc.)**

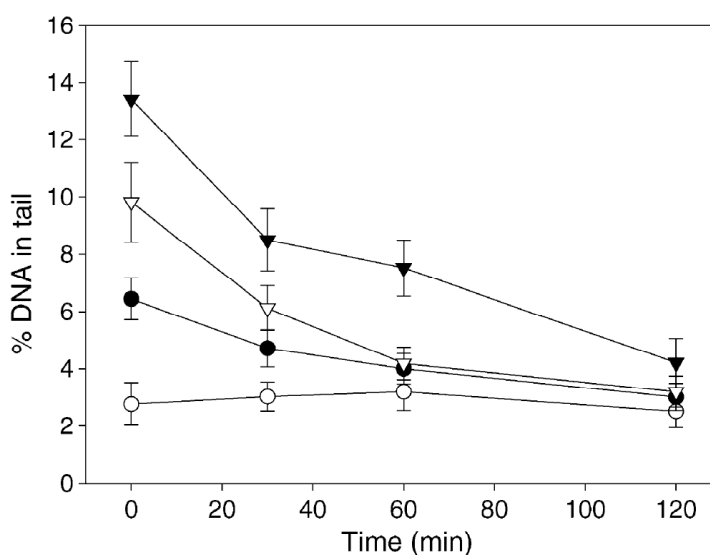


From Jenkins *et al.*, 2005

More specifically, for acrylamide there are 2 bases for a threshold. Firstly, acrylamide adducts are rapidly repaired by DNA repair enzymes (Blasiak *et al.*, 2004). As can be seen in Figure 3, DNA from human lymphocytes is repaired within the 60 minutes incubation. It is likely that at the enormous doses used in acrylamide mutagenicity assays, this system is overwhelmed. Secondly,

the depurination lesions and consequent chromosomal events are second order kinetics (Adler *et al.*, 1994) and have a threshold at low doses. There are metabolic considerations where one would expect the reaction with sulfhydryl groups and haemoglobin to vastly predominate over DNA reactivity, leaving little material to bind to DNA. Glutathione depletion may be a necessary event in acrylamide genotoxicity (Klaunig and Kamendulis, 2005; Yousef and El-Demerdash, 2006). These are discussed in detail below.

**Figure 3 : Time course of the repair of human lymphocytes**



From Blasiak *et al.*, 2004

Time course of the repair of DNA damage in human lymphocytes after incubation with acrylamide at 0.5  $\mu$ M (●); 5  $\mu$ M (▽) and 50  $\mu$ M (▼) as compared with untreated control: (○). The number of cells analysed in each treatment was 100. The data presented are mean results from three independent experiments; error bars denote S.E.M.

## **2. DNA Breaks are unrelated to sites of alkylation and rapidly repaired**

Treatment of 8-week old rats with 15 mg/kg of acrylamide in drinking water induced no increase in DNA strand breaks in liver (Klaunig and Kamendulis, 2005). In contrast, there was a significant increase in DNA breaks/labile sites in both the thyroid (50% over control) and adrenal (80% over control) glands as measured using the comet assay. The levels of DNA alkylation in all three organs are the same (Gamboa da Costa *et al.*, 2003; Fennell *et al.*, unpublished results). At a single oral dose of 54 mg/kg, only the brain and not the liver, bone

marrow, testes, or adrenals showed increase in comet response as measured by tail moment (Manière *et al.*, 2004). At 36 mg/kg and lower, no significant responses were observed. This is most likely due to oxidative stress, which will be discussed below (Yousef and El-Demerdash, 2006). In TK6 cells, acrylamide caused an increase in mutant frequency in the TK assay and MN test at a dose of 14mM while showing no DNA damage (Koyama *et al.*, 2006). In a loss of heterozygosity assay, this was accounted for by deletions. In V79 cells, acrylamide caused neither DNA damage, hprt mutations or micronuclei at concentrations of up to 6000 mM, 5000 mM or 10,000 mM, respectively (Baum *et al.*, 2005). The conclusion from all of these studies is that acrylamide alone is devoid of genotoxic activity. Glycidamide, on the other hand is a low potency mutagen. However, the involvement of glycidamide with DNA *in vivo* does not appear to account for all the data presented.

### **3. Prokaryotic cells are not mutated by acrylamide**

Acrylamide has been extensively tested with and without metabolic activation at up to 10 mg/plate in Salmonella and is not mutagenic at up to 5 mg/plate in one study (Emmert *et al.*, 2006), and was considered equivocal when tested at up to 10 mg/plate (Zeiger *et al.*, 1987), despite the fact that its metabolite, glycidamide, is mutagenic at 50 µg/plate (Hashimoto and Tanii, 1985). One possible explanation is that acrylamide is not converted to glycidamide *in vitro*. Emmert *et al.* (2006) transfected a methyl deficient strain of *S. typhimurium* with pin3ERb<sub>5</sub>. This plasmid contains cytochrome P450 reductase, cytochrome b<sub>5</sub> and cytochrome P450 2E1. Allyl chloride, and 2-aminoanthracene were mutagenic in this strain. Acrylamide at doses up to 10 mg/plate was not mutagenic in this strain showing that the prokaryote did not respond even when conversion to glycidamide took place. No satisfactory explanation for this phenomenon has been proposed.

### **4. Induction only of chromosome aberrations in mammalian cells in culture**

Acrylamide was clastogenic to L5178Y and TK6 cells and did not produce point mutations when tested without S-9 (Koyama *et al.*, 2006; Moore *et al.*, 1987). It was not tested with S9 in L5178Y cells. Measurement of colony size revealed that acrylamide induced only small colonies

which are indicative of chromosome aberrations and not point mutations. This conclusion was confirmed with cytogenetic analysis (Koyama *et al.*, 2006). An unpublished NTP study of acrylamide in CHO cells resulted in induction of chromosome aberrations with S9; the response without S9 was judged "questionable" (NTP, 2007). Results showing chromosomal aberrations and mitotic spindle effects, and not point mutation, are a recurring theme in acrylamide genetic toxicology (Davis and Recio, 2007; Koyama *et al.*, 2006). These lesions are of a threshold nature (Foth *et al.*, 2005; Bolt, 2003).

## **5. Acrylamide inhibits kinesin and causes oxidative stress *in vivo***

The most sensitive genetic toxicological endpoints for acrylamide effects are kinesin inhibition (Sickles *et al.*, 2007) and oxidative stress (Yousef and El-Demerdash, 2006). At a concentration of 100  $\mu\text{M}$ , acrylamide induces more than 50% inhibition of KIFC5A and KRP2 in rats. These enzymes centre and then segregate the chromosomes and then depolymerise the mitotic spindles. Inhibition of KIFC5A is consistent with the mitotic inhibition and the aneuploidy observed *in vitro* in fibrosarcoma cells (Sickles *et al.*, 1995). In the case of KRP2, glycidamide was 5- to 10-fold more potent than acrylamide. This difference is extremely relevant to explaining some of the differential sensitivities to glycidamide. That is, in the P4502E1 knock out mice, the lack of mutagenic activity may be dose related since acrylamide is much less potent than glycidamide. Furthermore, this same series of proteins is responsible for sperm motility and may explain some reproductive observations.

*In vivo* doses as low as 250  $\mu\text{g}/\text{kg}$  in rats produced statistically significant increases in oxidative stress effects as measured by increased thiobarbituric acid reactive-substances, and significantly reduced the content of sulfhydryl groups (Yousef and El-Demerdash, 2006). The highest dose tested was 500  $\mu\text{g}/\text{kg}$  while most *in vivo* genetic toxicology tests have been conducted at 100 to 200 times this dose. This shows that glutathione transferase increases after prolonged acrylamide exposure, suggesting another reason for a threshold effect. These effects were small but indicative of a potentially very large effect at the doses used in genetic toxicity studies which are generally 100-200 times greater. Even at these doses, the authors indicated that oxidative stress would be a basis for the organ effects that were seen.

Incubation of human lymphocytes with acrylamide resulted in a dose related increase in DNA in the comet tail over a range of 0.5  $\mu\text{M}$  to 50  $\mu\text{M}$  (Blasiak *et al.*, 2005). Post-treatment of the damaged DNA with repair enzymes: thymine glycol DNA *N*-glycosylase (Nth) and formamidopyrimidine–DNA glycosylase (Fpg), recognizing oxidized DNA bases, as well as 3-methyladenine–DNA glycosylase II (Alk A), recognizing alkylated bases, caused an increase in the expression of DNA damage, indicating the induction of oxidative and alkylative DNA base modifications by acrylamide. Pre-treatment of the lymphocytes with *N-tert*-butyl-phenylnitron (PBN), a free-radical spin trap, as well as vitamins C and E decreased the DNA-damaging effect of acrylamide, which suggests that free radicals/reactive oxygen species may be involved in this effect.

#### **6. The Big Blue Mouse data confirm that frameshifts, rather than base-pair substitutions, are the major component of the increased mutagenic response *in vivo***

One study was conducted on acrylamide and glycidamide using the Big Blue mouse showing weak induction of mutations of the *cII* gene (Manjanatha *et al.*, 2006), and two studies with acrylamide at the *lacZ* gene in the MutaMouse, only one of which was positive (Hoorn *et al.*, 1993; Krebs and Favor, 1997). There have also been two studies conducted in Big Blue mouse embryonic fibroblasts in culture (Besaratina and Pfeifer, 2003; 2004). The Big Blue studies all showed a weak induction of mutations, and conflicting results were obtained in the MutaMouse studies. The *in vivo* and *in vitro* Big Blue studies were the only ones to analyze and characterize the control and induced mutants, and these analyses are addressed below.

Acrylamide was tested *po* in male and female mice at 100 ppm (~25 mg/kg) for 4 weeks or 500 ppm (~100 mg/kg) for 3 weeks (study terminated due to toxicity), in parallel with equimolar doses of glycidamide. Mutation was evaluated at the *cII* and HGPRT loci, and the frequencies of micronucleated PCE in peripheral blood were also measured (Manjanatha *et al.*, 2006). The results showed that the micronucleus and HGPRT assays, both of which are responsive to substances that induce deletions and chromosomal effects, are more sensitive, in terms of fold-increases than the *cII* mutation assay (see Table 1). Because of the relatively small size of the *cII* gene, it is considered to be ineffective for detecting deletions, which may be one reason for its apparent reduced sensitivity. The *cII* mutant frequency was increased over the control by

approximately 2.1- and 2.3-fold for acrylamide and glycidamide, respectively, compared to the approx. 20- and 25-fold increases in HGPRT mutation frequencies induced by acrylamide and glycidamide, respectively.

When the acrylamide and glycidamide *cII* mutations at the high doses were sequenced and compared to the control animal mutations, there was a 58 and 60% decrease in transition mutations in the acrylamide and glycidamide treated mice, respectively. At the same time the acrylamide and glycidamide transversion mutations increased 65 and 69% above control, respectively, and frameshift mutations increased 84% above control with both chemicals (Table 1a). Because approximately half of the frameshift mutations occurred at a –GGGGGG– hotspot, the mutation fractions were recalculated without considering frameshift mutations (Table 1b). In this subsequent analysis, transition mutations decreased by more than half following chemical treatment, and transversion mutations doubled, essentially offsetting each other, with the decrease in transitions being greater than the increase in transversions. This suggests that the increase in frameshift mutations was a prime contributor to the overall increase in mutant frequency following acrylamide and glycidamide administration.

These frameshifts can induce the deletions which were observed in the other assays. It is noteworthy that glycidamide was not more mutagenic than acrylamide, although it is anticipated that only 50% of the acrylamide dose would be converted to glycidamide in the mouse, and glycidamide would be more effective at adducting DNA than acrylamide. There are no data in the literature to determine the effects of the peripheral neurotoxicity which were observed in this study on the *CII* results.

The other Big Blue mutation studies used mouse embryo cell cultures (Besaratina and Pfeifer, 2003; 2004). In these studies, cells were treated with a range of acrylamide concentrations from 32 nM to 320 mM (Besaratina and Pfeifer, 2003). Concentrations above 320 μM were cytotoxic. Mutants induced at 320 μM were sequenced and the pattern of DNA adducts in the *cII* gene was mapped, although not identified. It was concluded that acrylamide was a weak mutagen, producing up to a two-fold increase in mutant frequency, and preferential increases in some (*i.e.*, G>C) transversions and some (*i.e.*, A>G) transitions. There was no increase in frameshift frequency reported, but the authors elected not to consider mutations at

the –GGGGGG– hotspot. Equal numbers of these mutations appear to have been present among control and acrylamide-treated mutants. The authors also concluded that “there was no direct relationship between the pattern of induced mutations and the mapping of DNA adducts.”

A second study treated the cells with a range of 50 nM to 5 mM acrylamide and glycidamide, and the adducts in the *cII* gene were mapped (Besaratina and Pfeifer, 2004). As in the previous experiment, frameshift mutations at the –GGGGGG– hotspot were not included in the analyses. Glycidamide was mutagenic and induced an approximate 5-fold increase in mutant frequency at 500  $\mu$ M. Glycidamide treatment at 500  $\mu$ M produced increases in A>G transitions, and G>C and G>T transversions. The frequency of frameshift mutations not at the hotspot was decreased by glycidamide. The authors also concluded that, unlike acrylamide, the glycidamide mutations coincided with the sites of adduct formation.

Although all sets of experiments showed that acrylamide and glycidamide are mutagenic at the *cII* locus, the major difference between the *in situ* liver and fibroblast cell culture responses appear to reside in the differential induction of frameshift mutations, both at the hotspot and at other sites, *in vivo*.

Table 1 : Summary of genetic effects in the Big Blue mouse (from Manjanatha <i>et al.</i> , 2006) <sup>1</sup>					
Dose (mg/kg)	% MN-RET	HGPRT MF <sup>2</sup>		<i>cII</i> MF <sup>2</sup>	
	males	males	females	males	females
0	0.28 ± 0.02	2.2 ± 0.3	1.5 ± 0.5	28.4 ± 3.1	26.5 ± 4.5
AA19	NR	11.0 ± 2.9*	6.6 ± 1.0*	21.0 ± 2.6	26.0 ± 2.8
AA98 <sup>3</sup>	0.93 ± 0.13	41.0 ± 5.6**	32.6 ± 9.5**	57.2 ± 2.2*	59.0 ± 6.5*
GA25	NR	12.6 ± 2.9*	9.5 ± 3.1*	23.5 ± 5.8	33.5 ± 6.2
GA98	0.65 ± 0.07	33.6 ± 3.7**	51.0 ± 17.5**	67.2 ± 12.8*	60.2 ± 7.2*

<sup>1</sup> All data are mean ± S.D.

<sup>2</sup> MF x 10<sup>-6</sup>

<sup>3</sup> Animals exposed for only 3 weeks; all other dose groups were exposed for 4 weeks

NR Not reported

\* p ≤ 0.05; \*\* p ≤ 0.01

Table 1a : Analysis of <i>cII</i> mutations induced in the Big Blue mouse (from Manjanatha et al., 2006) when the frameshift mutations are included in the total						
	Transition mutations		Transversion mutations		Frameshift mutations <sup>1</sup>	
Dose (mg/kg)	% of total	Fold change	% of total	Fold change	% of total	Fold change
0	52	–	29	–	19 (9)	–
AA98	17	0.33	48	1.65	35 (18)	1.84
GA98	15	0.29	49	1.69	35 (20)	1.84

<sup>1</sup> percent in brackets are the % frameshifts at the –GGGGG– hotspot

Table 1b : Analysis of <i>cII</i> mutations induced in the Big Blue mouse (from Manjanatha et al., 2006) when the frameshift mutations are not included				
	Transition mutations		Transversion mutations	
Dose (mg/kg)	% of total	Fold change	% of total	Fold change
0	64	–	36	–
AA98	27	0.42	73	2.03
GA98	24	0.40	76	2.11

## 7. The data reveal a threshold in the micronucleus test *in vivo*

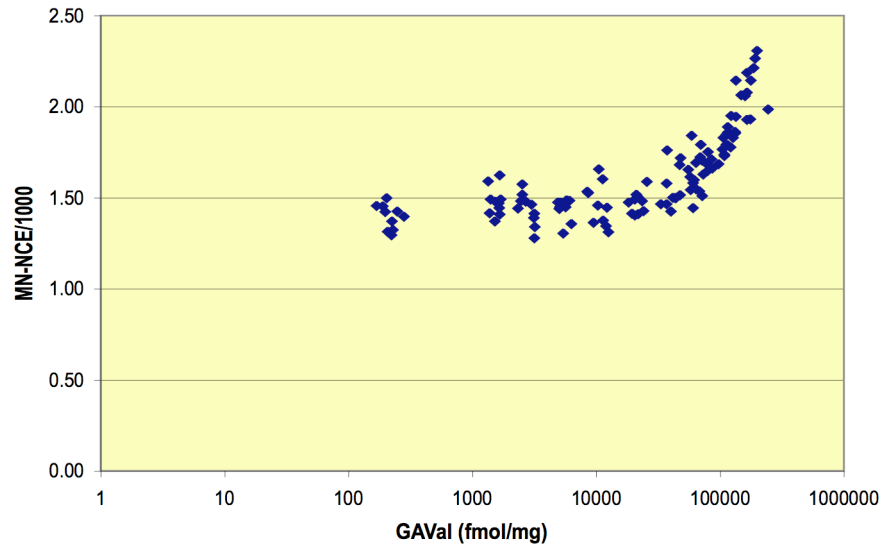
Abramsson-Zetterberg (2003) concluded that the micronucleus response was consistent with the lack of a threshold, although the data presented did not allow a full statistical evaluation of the response. To further investigate this, a comprehensive study was conducted to determine the shape of the dose–response curve in the low-dose range in the mouse micronucleus test. This study was planned by a committee of toxicologists and statisticians to lend itself to statistical analysis at its completion. A large, 4–week gavage study was conducted to GLP with 11 doses of acrylamide ranging from 0.125 mg/kg to 25 mg/kg and 10 male mice per group. The highest

daily dose, which was given for 28 days, was approximately 23% of the single dose oral LD<sub>50</sub>. Peripheral blood MN-PCEs and MN-NCEs were determined by flow cytometry and approximately 2\*10<sup>5</sup> MN-PCEs and 10<sup>6</sup> MN-NCEs per mouse were assayed to minimize assay variance and provide additional statistical power. Data were corrected for internal dose using both acrylamide and glycidamide haemoglobin adducts.

The response was relatively weak with only a 1.4-fold increase in MN-PCE. The NOAEL for MN-PCEs in this study was 4.0 mg/kg/day. A non-linear response was not apparent in the PCEs as the variability in this response was much greater than that in the NCEs (Haseman, report in preparation). Both linear and non-linear models fit these data equally well. In the case of NCEs, both models also fit equally well when linear dose was used as the metric. However, when log dose was used as the metric, the response with the nonlinear model was a better fit. Correcting the data using GAVAL (glycidamide Hb adducts) as the dose metric produced a clear and statistically significant nonlinear response. The data were more consistent with a threshold than a linear extrapolation, and significant fits were observed at a threshold at 2 mg/kg.

As shown in Figure 4 below, when the incidence of micronuclei was corrected for GAVAL – the dose metric for the mutagenic metabolite – the consequent dose-response curve showed significant ( $p < 0.01$ ) non-linearity. One way to account for this non-linearity is with a threshold-based model assuming a threshold dose of 2 mg/kg. Using GAVal as the dose metric reduced the significance of the linear regression ( $F=585.26$ ;  $r\text{-squared}=0.833$ ) compared with using administered dose. The threshold model provides an excellent fit to the data and a better fit than a linear model. The departure from linearity was statistically significant ( $p=0.002$ ). Because of this, the threshold regressions showed a better fit to the data than the linear model fit: Threshold dose=1,  $F=687.61$ ,  $r\text{-square}=.856$ ; Threshold dose=2,  $F=669.46$ ,  $r\text{-square}=0.852$ .

Figure 4 : Micronuclei vs GAVAL \*

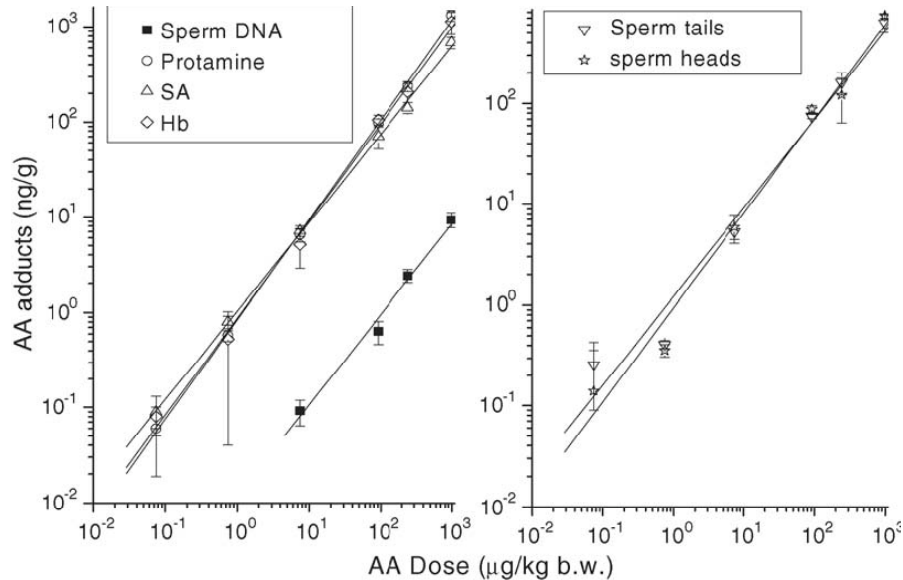


\* Individual animal data are plotted

## 8. Acrylamide binds to protamines *in vivo*

There are no informative studies on binding of acrylamide or glycidamide to sperm DNA. Both acrylamide and glycidamide are vastly more reactive with proteins than DNA. All the studies on sperm DNA in the literature used radioactive acrylamide and DNA contamination with protein was likely (Xie *et al.*, 2005). Only a minute amount of protein would be needed to contaminate the DNA and alter the interpretation of the results of the studies.

**Figure 5 : Dose-Responses of bio-macromolecule adducts with acrylamide**



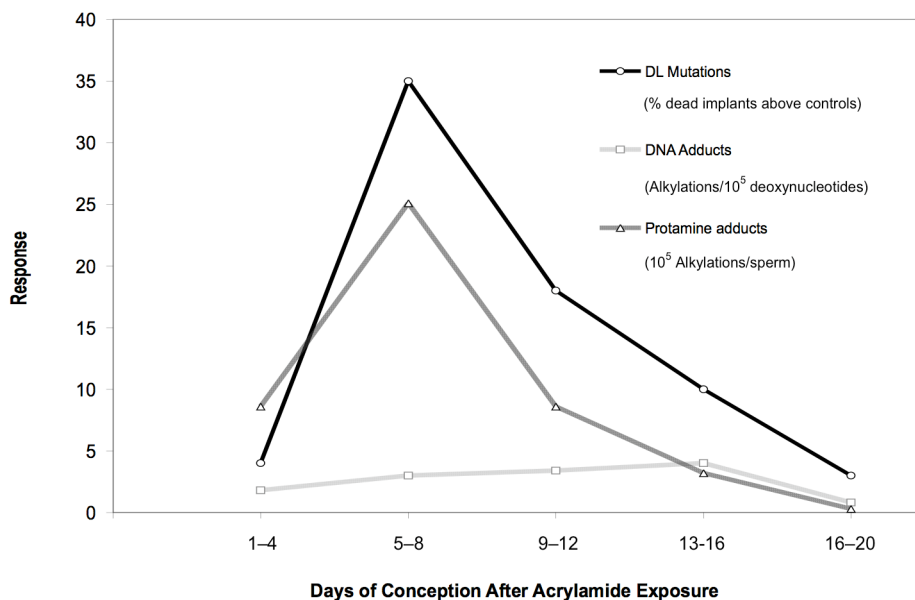
From Xie *et al.*, 2005

The linear fitting equations are (where  $r$  is the correlation coefficient): AA-sperm DNA,  $\lg Y = 0.95 \lg X - 1.92$ ,  $r = 0.9939$ ; AA-protamine,  $\lg Y = 1.05 \lg X - 0.08$ ,  $r = 0.9997$ ; AA-SA,  $\lg Y = 0.93 \lg X + 0.01$ ,  $r = 0.9996$ ; AA-Hb,  $\lg Y = 1.02 \lg X - 0.06$ ,  $r = 0.9982$ ; AA-sperm tails,  $\lg Y = 0.88 \lg X + 0.08$ ,  $r = 0.9868$ ; AA-sperm heads,  $\lg Y = 0.93 \lg X - 0.02$ ,  $r = 0.9919$ . Values represent means  $\pm$  S.D. ( $n = 4$ ).

There is a chronological correlation between alkylation of protamines and induction of dominant lethal mutations while DNA alkylation appears constant (Sega *et al.*, 1989). In Figure 6 below, a chronological comparison is presented between dominant lethal mutations (DL), DNA adducts, and protamine alkylation. As can be seen, dominant lethal mutations and protamine alkylation both peak at conception day 8. In contrast, DNA alkylation is minimal and constant throughout the time period, consistent with data from Manière *et al.* (2005) who also showed early and constant DNA alkylation. The dominant lethal mutations correlate very well with the protamine alkylation, but have no correlation with DNA adducts. This strongly suggests that the protamine binding is substantially more important than the DNA binding for induction of dominant lethals. What is needed is isolation of epididymal DNA. However, based on current data, DNA alkylation does not appear related to mutational events seen with acrylamide. Since DNA alkylation is measured by radioactivity from administered acrylamide, it does not distinguish which reaction product it represents. Given that protein is more plentiful than DNA and more

highly reactive with acrylamide, especially protamines, slight contamination with protein would appear as significant DNA alkylation. “The mechanism of toxicity of acrylamide to the genetic material of male germ cells is correlated with protamine binding, possibly by glycidamide rather than by acrylamide itself, with DNA breakage secondary to stresses imposed on the chromatin by protamine binding” (NTP, 2004).

**Figure 6 : Comparison of DNA and sperm alkylation with dominant lethal mutations**



From Sega *et al.*, 1989

Adler *et al.*, (2000) inhibited cytochrome P4502E1 to block glycidamide production which inhibited dominant lethality. In this study, micronuclei were assayed but the induction was not statistically significant in the acrylamide alone group (unpublished results). These results are a likely reflection of the relative protein reactivity between glycidamide and acrylamide and not DNA reactivity. The relative protein reactivity was comparable to the percentage inhibition of dominant lethality.

## 9. The induction of heritable translocations is non-linear

Acrylamide induces heritable translocations in mouse sperm (Adler *et al.*, 1994). Induction of these chromosomal anomalies fits a binominal distribution; probably like all chromosomal anomalies. This is due to the requirement for at least two hits in close proximity. This parabolic distribution shows that mutagenicity studies at high doses enormously overestimate risk. Statistical analysis of these data reveal a doubling dose of 3 mg/kg demonstrating a virtual threshold at 3.7 mg/kg. It can be assumed that humans and mice do not show equivalent since mice produce substantially more glycidamide than humans. Nevertheless, the conservative assumption of equal sensitivity reveals that at 3.7 mg/kg, the response would be well within background and virtually nonexistent.

## 10. Acrylamide is only a very weak clastogen *in vivo*

Treatment with the maximally tolerated dose for 28 days only resulted in less than a doubling of micronucleated red blood cells in mice. The benchmark doses for genetic toxicity, which represents the upper bound confidence limits for the 10% response based on curve fitting, have been calculated (see Table 2 below) and are on the order of 50% of the LD<sub>50</sub> (Allen *et al.*, 2005). The high BMD is reflective of the weak activity of acrylamide in these tests. By both measures of intrinsic activity (how mutagenic is it) and potency (at what doses do the mutations occur), acrylamide is a weak mutagen.

Table 2 : Mutagenic Potency of Acrylamide in Rodent Cells <i>In Vivo</i>		
Cell Type	Benchmark Dose (BMD) <sup>1</sup>	BMD as % of LD <sub>50</sub>
Mouse somatic cell	43 mg/kg	40
Mouse germ cell	59 mg/kg	55
Rat somatic cell	111 mg/kg	88
Rat germ cell	Inactive	NA

<sup>1</sup> From Allen *et al.*, 2005

## **Conclusion**

Acrylamide is a murine aneugen and clastogen causing chromosome damage in bone marrow and sperm cell damage in rodents. It is extremely weak, albeit consistently active. The genetic effects appear to be more dependent on its protein reactivity than on its DNA reactivity.

The micronucleus dose response in NCEs is consistent with a NOAEL of 4 mg/kg for the reticulocytes and a mathematically calculated threshold at 2 mg/kg/day for the normochromatic-erythrocytes. That is, at doses of 2 mg/kg/day and below, acrylamide has no genotoxic effects *in vivo* in the mouse. In humans, this threshold would be expected to be much higher since humans metabolise acrylamide to glycidamide at a substantially lower rate. The reactivity of acrylamide with kinesin suggests that non-disjunction may contribute to the induction of micronuclei.

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