THE AN GROUP

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TSCA Confidential Business Information Center (7407M)
EPA East - Room 6428 Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Contain NO CBI

Re: Genetic Toxicology Information on Acrylonitrile (CAS No. 107-13-1)

Dear TSCA 8(e) Coordinator:

On behalf of The Acrylonitrile Group (AN Group) and its members,[1] I am writing to notify the Agency of the following information which was recently provided to the AN Group by Dr. Vernon Walker. These studies were conducted by Dr. Walker in collaboration with other researchers. Dr. Walker is currently at Lovelace Respiratory Research Institute in Albuquerque, NM, and provided this information in response to an expert panel review of acrylonitrile health effects information sponsored by the AN Group. The significance, if any, of this information to any potential human health risks associated with acrylonitrile exposure has not been determined.

Study A: *Series of three in vivo Hprt genetic toxicity studies in female F344 rats.*

The *in vivo* mutagenicity of acrylonitrile (AN) was assessed at the hypoxanthine guanine phosphoribosyl transferase (*Hprt*) locus in T-lymphocytes in a series of three overlapping experiments conducted using drinking water exposures of F344 rats to: (a) determine the effect of exposure duration on the frequency of *Hprt* mutant T-cells [Mf]; (b) characterize the effect of time elapsed after AN exposure on *Hprt* Mf in splenic and thymic T-cells; and (c) define the dose-response for *Hprt* Mf in splenic T-cells.

The three experiments and a brief statement of their results follows:

Experment 1: Groups of rats (4 weeks old) were exposed to 0 or 500 ppm AN for up to 4 weeks and necropsied 2 weeks after the cessation of exposure.

Results: In both thymus and spleen, exposure to 500 ppm AN over time led to an increased *Hprt* Mf that was directly related to the duration of exposure.

[1] Members of the AN Group include: Cytec Industries Inc.; The Dow Chemical Company; DuPont Company; INEOS Nitriles; Lanxess Corporation; SABIC Innovative Plastics and Solutia Inc.

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Experiment 2: In order to characterize the effect of time elapsed after AN exposure on *Hprt* Mfs in splenic and thymic T-cells, groups of rats (n=3 control and 5 treated/dose group) were necropsied at 0, 2, 4, 6 or 8 weeks after 4 weeks of exposure to 0 or 500 ppm AN.

Results: *Hprt* Mf in the thymus of exposed rats reached a maximum 2 weeks post exposure (i.e., 6.1 ± 0.5 SE x 10^{-6}, versus control values of $1.9 \pm 0.2 \times 10^{-6}$) and then decreased to control levels. In splenic T-cells of exposed rats, *Hprt* Mf were significantly elevated at the end of the 4 week exposure period, increasing in a linear fashion to reach a maximum 4 weeks post exposure (i.e., Mf of $11.1 \pm 2.7 \times 10^{-6}$, versus a background Mf of $2.6 \pm 0.4 \times 10^{-6}$), and then declined to lower but still elevated levels.

Experiment 3: To define the dose-response for *Hprt* Mfs in splenic T-cells, groups of rats (n=3 control and 5 treated/dose group) were necropsied 4 weeks after exposure to 0, 33, 100 or 500 ppm AN. At necropsy, T-cells were isolated from thymus and/or spleen and cultured to recover *Hprt* mutant colonies.

Results: Exposures to 100 and 500 ppm AN (average daily doses of 21 and 76 mg AN/kg body weight/day, respectively) led to significant increases in Mf that were 2.0- and 4.3-fold, respectively, above background.

Study B: *In vivo Hprt genetic toxicity study in B6C3F1 wild type mice and CYP2E1-null mice.*

The *in vivo* mutagenicity of acrylonitrile (AN) was assessed at the hypoxanthine guanine phosphoribosyl transferase (*Hprt*) locus in T-lymphocytes in a series of studies in rats or mice. A study was conducted in the spleen of female wild-type versus CYP2E1-null mice given daily doses of AN by gavage for 4 weeks.

The *Hprt* Mf of T-cells in spleens of female B6C3F1wild-type mice versus CYP2E1-null mice were examined following daily doses of AN by gavage for 4 weeks, measured 24 hours after the last dose, at 0, 2.5, 10 or 20 mg/kg/day for wild type and 0, 20 or 60 mg/kg/day for CYP2E1-null mice.

The average observed *Hprt* Mf ($\times 10^{-6}$) of T-cells in spleens of wild type mice given 0, 2.5, 10 or 20 mg/kg/day was 1.7 ± 0.9, 2.3 ± 1.9, 3.1 ± 1.2 or 5.4 ± 2.4 (statistically signifcant P-Value 0.016), respectively. The average observed Mf ($\times 10^{-6}$) for CYP2E1-null mice given 0, 20 or 60 mg/kg/day was 1.7 ± 0.9, 1.6 ± 0.7 or 5.3 ± 2.3 (statistically signifcant P-Value 0.002), respectively.

Study C: *In Vitro DNA Adduct Study.*

The formation of DNA adducts after *in vitro* treatment of DNA isolated from brain or liver of naïve control rats with the epoxide metabolite of acrylonitrile (2-cyanoethylene oxide; CEO) was assessed.

Results: Treatment of brain or liver DNA from control rats with CEO [AN epoxide] resulted in significant formation of OEG [7-2-oxoethylguanine] *in vitro*, with a linear dose response of similar slope observed in DNA from both tissues. Barbin *et al.*[2] have noted that 7-(2-oxoethyl)guanine does not miscode for either thymine or adenine, thus supporting the contention that it is not a pro-mutagenic adduct.

Study D: *In Vivo DNA Adduct Study.*

The formation of DNA adducts as assessed after *in vivo* exposure to acrylonitrile (AN) in drinking water.

Results: Analyses for OEG adducts in liver DNA from rats exposed to 500 ppm AN for 15 months or to 300 ppm AN for 3, 7, 14, 28 or 105 days revealed the presence of peaks equivalent to less than the acceptable detection limit for this adduct (i.e., 7 pmol OEG/mg DNA at a signal to noise ratio of 10:1). Similar peaks were not seen in liver DNA from control rats.

Study E: *Comet Assay.*

DNA damage was measured in forestomach and liver cells of B6C3F1 mice administered acrylonitrile (AN) by gavage daily for 4 weeks.

Results: The Olive Tail Moment (OTM - defined as the fraction of migrated DNA in the tail x the geometric mean of the tail) was significantly increased ($p<0.05$) in mice administered 2.5 mg/kg AN (forestomach DNA), and 10 or 20 mg/kg AN (forestomach and liver DNA) as compared to untreated control animals. Mean OTM values in forestomach were 2.5 ± 0.28, 6.4 ± 1.02, 5.3 ± 0.76, and 5.5 ± 0.68, and 2.4 ± 0.35, 2.9 ± 0.51, 3.8 ± 0.67, and 5.9 ± 0.71 in liver (control to high dose).

Please do not hesitate to contact me if we can provide any further information.

Sincerely,

Robert J. Fensterheim
Executive Director

[2] Barbin, A., Laib, R.J., and Bartsch, H. (1985, June). Lack of Miscoding Properties of 7-(2-Oxoethyl)guanine, the Major Vinyl Chloride-DNA Adduct. Cancer Research, 45, 2440-2444.