Can Anionic Polymers used for Gel Flocculants break down into Acrylamide Monomers?

Chemicals present in Clear Flow's Soil Lynx, Water Lynx and Gel Flocculant polymer products meet ANSI/NSF Standard 60 Drinking water treatment chemical standards and the anionic PAM within the products contains no more than 0.05% wt/wt residual AMD. This is a very low concentration of AMD and is suitable for potable water treatment (resulting in drinking water AMD concentrations not exceeding 0.5 μg/L when used at an appropriate concentration; U.S. EPA National Primary Drinking Water Regulations). We do not intend for the treatment of potable water, but it is important to note that the amount of residual AMD in our product is controlled and not allowed to exceed a maximum value. Anionic PAM only makes up approximately 40% of the Gel Flocculant. This further reduces the maximum amount of AMD present in each gram of Clearflow's Gel Flocculant.

Polyacrylamides do not break down into AMD or other known toxic compounds under environmental conditions (as reviewed by Caulfield *et al.,* 2002). Bacteria have been shown to be capable of growth using PAM as a sole source of nitrogen, but not as a sole source of carbon (Kay-Shoemake *et al.*, 1998b). Since the same bacteria were shown to be able to survive off of acrylamide as a sole source of both nitrogen and carbon (Kay-Shoemake *et al.*, 1998b) this indicates that the bacteria were unable to break down PAM into acrylamide monomers. While physical force and ultraviolet radiation can break the linear chain of PAM into smaller polyacrylates of various chain lengths (Barvenik, 1994), polyacrylate chains of ≤ 10 000 g/M have been shown to be able to be ultimately broken down into CO_2 and H_2O by the process of mineralization (Kawai, 1993).

Regarding the potential risk of AMD leaching from PAM treated fields into groundwater, a recent scientific study (Lentz *et al,* 2008) has investigated this subject. Lentz *et al.* (2008) conducted added anionic PAM to irrigation water at a concentration of 10 mg/L that then flowed into irrigation canals. They then measuring residual AMD concentrations in percolation water and furrow inflows following three furrow irrigations. The experiment occurred on a long-term field plot experiment near Kimberly, ID that had had PAM treatments in continual operation since 1993. The PAM that was used claimed to contain <0.05% wt/wt of residual AMD monomer; however, following analysis of the PAM product the authors discovered that it only contained 0.018% wt/wt AMD. Consequently, the authors created a 'worst case scenario' by adding raw AMD to the PAM stock solution until it constituted 0.05% wt/wt. While the AMD concentration in the furrow inflows was the expected 5 μg/L value (based upon a 0.05% wt/wt value), AMD concentrations within percolation water samples during all post-irrigation periods and all irrigations were below the minimal detection limit (MDL of 0.5 μg/L; Lentz et al 2008). The authors came to the conclusion that this reduction in AMD concentration was due to both dilution and microbial degradation. They also state that "the risk that ground water beneath these water-soluble PAM-treated furrow irrigated soils will be contaminated with AMD appears minimal". Lastly, Lentz et al (2008) also noted that since much of the potable water-grade PAM products used in erosion control and irrigation contains about half the allowable AMD, this would further reduce the risks of possible contamination.

No Reports of Ecological Toxicity From AMD Have Been Associated with Anionic PAM Use

Anionic PAMs used by Clear Flow are fully polymerized with <0.05% AMD monomer; thus there will be no widespread release of significant amounts of AMD to the environment.

In order to reach concentrations which cause the listed toxic effects on the fish and cattle (500 mg/L, or 0.5 g/L) the PAM concentration would have to be 1,000,000 mg/L. That is 1 kg/L polymer. Information provided by Clear Flow's manufacturer indicates that Gel Flocculant products only release at between 0.01mg/L up to a high of 5 mg/L. Third party testing was conducted at the University of Alberta Department of Biosciences laboratories where the Gel Flocculant was placed in a centrifuge for 72 hours to see what a forced release would indicate. The results of this centrifuge tests were a maximum release rate of 25 mg/l. The Gel Flocculants cannot be made into a fully soluble stock solution(Kerr et al 2014) In another sample calculation (using data from an *in-situ* site) information indicated that the average Gel Flocculant polymer concentration over a 7-day pumping period (568 L/min) was 2.1 mg/L. These Gel Flocculant concentrations are between **40,000** and **10,000,000** times lower than the 100,000 mg/L concentration that the authors state would be required to cause the AMD poisoning in exposed organisms. Personal experiences of Clear Flow staff has shown that it is extremely difficult to create high concentration stock solutions of Gel Flocculant products – the products simply will not dissolve when a mass (e.g. 1000 mg) is placed in a set volume of water (e.g. 1L) and allowed to mix for >48 hrs.

In the case of Clearflow's Granular products (Soil Lynx, Lynx Ultra Bind, and Water Lynx Granular) high concentration solutions are possible, but only to ~ 3000 mg/L. Experience has shown that any Granular concentrations higher than this will not fully dissolve even after 48-72 hrs of mixing. Granular stock solutions of 3000 mg/L concentration are also extremely viscous. In fact, Clear Flow staff conducted trial calculations to see if it would even be possible for Granular products to be spilled into a stream and have AMD cause toxicity issues.

For example: If two 23 kg (50 pound) bags of Granular (total weight = 45.4 kg or 100 lbs) were accidentally dumped into a stream system, they would have to become fully dissolved in 45.4 liters of water in order to result in a residual AMD concentration of 500 mg/L (0.5 g/L), the concentration which occurred in the above case history.

$$45.4\text{kg PAM} \times 0.0005 = 22.7 \text{ g AMD}$$
$$22.7\text{g AMD} \div 0.5 \text{ g/L AMD} = 45.4 \text{ L water}$$

Quite simply, it is impossible to dissolve 45 kg of Granular in 45 L of water. The product would become a solid gel mass due to the hydroscopic nature of the anionic PAM. The only way this gel mass would fully dissolve is by having high volumes of fresh-water flow over top of it, in which case the concentration of PAM and any residual AMD would be reduced to very low levels.

We took this one step further by examining U.S. EPA's MSDS for acrylamide on the internet (http://www.epa.gov/chemfact/s_acryla.txt). Within the "Toxicity to Aquatic Organisms" section it lists the toxicity of AMD to various fish species. The most sensitive species, *Poecilia reticulata* (guppy) had a 7-day LC50 value of 35 mg/L AMD. We calculated that in order to reach these levels of residual AMD using our products, the concentration of PAM would have to be 70,000 mg/L (70,000 mg/L PAM ×0.0005 = 35 mg/L residual AMD). We created a 70,000 mg/L solution using Clearflow Granular products and hot water (7.0 g in 100 mL). Hot water was used because the Granular dissolves best in warmer water versus colder water, and we wanted to create a 'worst case scenario'. The resulting mixture was a solid gel mass that did not flow, proving that it would be impossible to create a situation in which fish were exposed to 35 mg/L residual AMD through the addition of the appropriate concentration of PAM.

If Clearflow Granular products were to ever be accidentally added in a ditch at this level, the product would create a gel block and certainly not be able to flow into a stream system and cause deleterious effects to aquatic organisms due to residual AMD concentrations. All these characteristics of the Clearflow Gel Flocculant and Granular products make it virtually impossible for PAM to reach the high concentrations required for residual AMD to become an issue, even if applicators were somehow uninformed and attempted a 'more is better' approach.

Bioaccumulation

Bioaccumulation is a major concern in environmental toxicology. However, due to the very large size of PAM molecules, this compound is unable to cross the cell membrane of tissues such as the gastrointestinal tract and thus is at low risk for bioaccumulation (Stephens, 1991). Indeed, repeated exposure-depuration fish studies involving several different types of polymers (epichlorhydrin-dimethylamine, polyacrylamide ester, and a polyacrylamide amide) did not result in bioaccumulation within gill tissue of exposed fish – the only tissue in which the polymers were found to bind in large quantities (Muir *et al.*, 1997). Binding was reversible.

Toxicity

Polyacrylamides have very low toxicity in mammalian systems (no significant adverse effects in rats exposed orally or dermally to > 5 mg/kg or to lower PAM concentrations in chronic toxicity studies, and no compound related lesions in a three generational study in rats; McCollister *et al.* 1965; Stephens, 1991).

Anionic polyacrylamides also have very low toxicity to aquatic life (Barvenik, 1994; Entry *et al*., 2002; Liber *et al*., 2005; Weston *et al.,* 2008). For example, Weston *et al.* (2009) recently published a study investigating the toxicity of anionic PAM formulations when used for erosion control in agriculture. They evaluated a number of different anionic PAM products for acute and/or chronic toxicity to aquatic amphipods (*Hyalella azteca*), midge larvae (*Chironomus dilutes*), water fleas (*Ceriodaphnia dubia),* fish *(Pimephales promelas*) and freshwater algae (*Selenastrum capricornutum*). Weston *et al.* (2009) found that with a granular PAM product (similar to Silt Stop) four of the five species tested showed no evidence of toxicity at even the highest concentration tested (100 mg/L) – only *C. dubia* showed an effect from granular PAM (reproductive IC50 of 5 mg/L and LC 50 of 29 mg/L). The authors state that "if it is assumed that sufficient granular material is used to achieve the same PAM concentrations desired when using liquid formulations (1-10 mg/L PAM), then it appears no acute toxicity to any of the test species would be expected, and at most there may be impairment of *C. dubia* reproductive ability within this concentration range". A tablet form of PAM (similar to Gel Flocculant) resulted in no indication of toxicity up to at least 100 mg/L to midge larvae (Weston *et al*., 2009). The authors also point out that laboratory testing represents worst-case conditions, partially because PAM adsorbs to soil and can be lost from tailwater in a relatively short distance after leaving the site of application (Lentz *et al*, 2002). As mentioned earlier, Clear Flow's Gel Flocculant products have been calculated to release at between 0.01 mg/L to 5 mg/L (data supplied by manufacturer).

In addition to the scientific literature available on anionic PAMs, Clear Flow has conducted numerous extensive toxicity tests on our own anionic PAM products (whole product toxicity; not simply the active ingredient). These toxicity tests have shown very low toxicities of both Clearflow's Gel Flocculant and Clearflow's Granular products to aquatic life. All acute and chronic toxicity tests were conducted by certified laboratories following government standard toxicity tests (Environment Canada and U.S. EPA) and are freely available upon request. In addition to government standard toxicity tests, Clear Flow has examined the effect of chronic, sub lethal polymer exposure on juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to different concentrations (3 mg/L – 300 mg/L) of each Clearflow Gel Flocculant and Clearflow Granular polymer product for up to 30 days. They were then sampled at either Day 7 or Day 30 post exposure and analysed for gill histopathology as well as elevation in an enzyme associated with oxidative stress. These studies were conducted at the University of Alberta and the University of Guelph under the supervision of a professor and funded by the National Research Council of Canada. Gill histopathology was analysed by a trained pathologist in a blind fashion.

Results indicated that in general, fish exposed to the majority of Clearflow Gel Flocculants and Clearflow Granular products had either no gill pathology or mild-to-moderate levels of pathology (depending upon the product examined and exposure concentration) compared to time matched controls. In many cases, pathology levels were reduced by the Day 30 sampling period compared to the Day 7 sampling period indicating that the anionic polymer may have induced a short-term irritant effect in the fish gill tissue. Rainbow trout fingerlings exposed to lower, environmentally relevant concentrations (3 mg/L, 30 mg/L) of Clearflow Gel Flocculants and experienced extremely low levels of gill pathology, even after 30 days of chronic exposure. In many cases the level of pathology observed in these fish did not differ from that of fish exposed to clean water controls. On the rare occasions when slight differences were observed the level of damage was very mild and likely not of biological significance (since very low to zero mortality was observed in all anionic polymer treatment groups even after 30 days of constant chronic exposure).

It should be noted that while some mild to moderate levels of histopathology were observed at the highest concentration of polymer tested, these concentrations are not expected to occur in 'real world' applications. The tests were done in clean water, free of suspended solids such as clay which normally binds with very high affinity to the anionic polymer products. In real world situations it is expected that the concentration of 'free polymer' in the water column would be far lower than the concentrations tested in this study. Furthermore, dilution effects in downstream environments would further reduce any residual, unbound anionic polymer molecules. Interestingly, similar levels of pathology were often seen in fish exposed a cationic polymer positive control run at the same time- even though the concentration of the cationic polymer was ~ *1000 times lower* than that required to see an effect with the anionic polymers. This 1000-fold difference in concentration emphases the toxicity of cationic polymers at very low concentrations and contrasts with that of anionic polymers, which require high concentrations induce gill histopathology effects.

For the enzyme associated with oxidative stress (glutathione s-transferase, GST), at no time point did exposure to the highest concentration of Clear Flow's anionic polymer products induce GST activity substantially higher than that of control fish. Since an increase in GST activity is generally regarded as a biomarker for oxidative stress, this would indicate that the Gel Flocculants and Granular products tested do not induce oxidative stress in rainbow trout gill tissue at concentrations ranging between 100 mg/L and 300 mg/L, as measured through elevated GST activity. These findings support the evidence that anionic polyacrylamides are unable to penetrate the cell membrane of aquatic organisms – the GST enzyme family are cytosol, mitochondrial and microsomal proteins. They aid in biotransforming xenobiotics such that they can be more easily excreted from a cell. Thus, if PAMs are unable to penetrate a cell membrane due to their large molecular size (as indicated by the scientific literature; Stephens, 1991), GST enzymes would not be required to assist in detoxifying the compounds and enzyme activity would not be increased following exposure to anionic PAMs in the environment. All data is available upon request.

References

Barvenik, F.W., 1994. Polyacyrlamide characteristics related to soil applications. Soil Sciences, **158**(4): 235-243

Caulfield, M.J., Qiao, G.G. and Soloman, D.H.. 2002. Some aspects of the properties and degradation of polyacrylamides. Chemical Reviews, **102**: 3067-3083.

Entry, J.A., Sojka, R.E., Watwood, M. and Ross, C., 2002. Polyacrylamide preparations for protection of water quality threatened by agricultural runoff contaminants. Environmental Pollution, **120**: 191-2000.

Kawai, F., 1993. Bacterial degradation of acrylic oligomers and polymers. Microbiology Biotechnology, **14**: 382-385.

Kay-Shoemake, J.L., Watwood, M.E., Lentz, R.D., Sojka, R.E., 1998a. Polyacrylamide as an organic nitrogen source for soil microorganisms with potential impact on inorganic soil nitrogen in agricultural soil. Soil Biology and Biochemistry, **30**: 1045-1052.

Kerr et al 2014, Effects of Anionic Polyacrylamide Products on Gill Histopathology in Juvenile Rainbow Trout, Enviro Tox and Chemistry pp.1-11

Lentz , R.D., R.E. Sojka, and B.E. Mackey. 2002. Fate and efficacy of polyacrylamide applied in furrow irrigation: Full-advance and continuous treatments. J. Environ. Qual. **31**: 661-670.

Lentz, R.D., Andrawes, F.F., Barvenik, F.W. and Koehn, A.C. 2008. Acrylamide monomer leaching from polyacrylamide-treated irrigation furrows. Journal of Environmental Quality, **37**: 2293-2298.

Liber, K., Weber, L. and Levesque, C., 2005. Sublethal toxicity of two wastewater treatment polymers to lake trout fry (*Salvelinus namaycush*). Chemosphere, **61**(8): 1123-1133.

McCollister, D.E., Hake, C.L., Sadek, S.E. and Rowe, V.K. 1965. Toxicologic investigations of polyacrylamides. Toxic. Appl. Pharmac., **7**(5): 639-651.

Muir, M. M., Kosteretz, K. G. and Lech, J. J., 1997. Localization, depuration, bioaccumulation and impairment of ion regulation associated with cationic polymer exposure in rainbow trout (*Oncorhynchus mykiss*). Xenobiotica, **27**(10): 1005-1014.

Reynolds, T. 2002. Acrylamide and cancer: tunnel leak in Sweden prompted studies. Journal of the National Cancer Institute, **94** (12): 876-878.

Stephens, S.H., 1991. Final report on the safety assessment of polyacrylamide. Journal of the American College of Toxicology, **10:** 193-202.

Weideborg, M., Källqvist, T., Ǿdegård, K.E., Sverdrum, L.E. and Vik, E.A. 2001. Environmental risk assessment of acrylamide and methylolacrylamide from a grouting agent used in the tunnel construction of Romeriksporten, Norway. Water Research, **35** (11): 2645-2652.

Weston, D.P., Lentz, R.D., Cahn, M.D., Ogle, R.S., Rothert, A.K., and Lydy, M.J. 2009. Toxicity of anionic polyacrylamide formulations when used for erosion control in agriculture. Journal of Environmental Quality, **38**: 238-247.