

From: Nina Wilson [REDACTED]
Sent: Wednesday, December 17, 2014 4:54 PM
To: Cooper, Arthur
Subject: ammonium acetate studies

Hi Dr Cooper;

Saw you at the last IRIS review and we chatted a bit about this and since I have looked at this issue for a few months. Below might be of interest to you and put this Satpute paper into context. The It seems that you cannot eat ammonia in such a large quantity and fast enough to cause hyperammonemia because the body metabolizes it so fast (your work!) so not sure how it is relevant to incidental oral exposure except in the case where you have in born error or severe liver disease in which case ammonium salt ingestion and possibility of toxic effects is the least of your problems.

I've spent the summer with ammonium salt lit. Below are some other ammonium acetate studies that I have found. It seemed that the Satpute reported dose does not fall in line with the other studies (calculated) of other experimental animal models. Ammonium acetate was used because they thought it was a less traumatic model using other salts where the accompanying anion resulted in conflating toxicity (Azorín, I., Miñana, M., Felipo, V., and Grisolia, S. (1989) A simple animal model of hyperammonemia. *Hepatology*.) What Satpute is seeing acetic acid trauma. Let me know what you think.

Chemically, AMA is a salt of a weak acid and a weak base and may be considered either an ammonium salt or an acetic acid salt. For toxicological consideration, however, the Agency for Toxic Substances Disease and Registry (ATSDR) demonstrate that reading across ammonium salts is not appropriate because of the influence of the anions.

It is, however, appropriate to read across end points from chemicals in the acetic acid salt group which includes AMA. At physiological pH, acetic acid fully ionizes to acetate, which is the common moiety of both AMA and acetic acid. The Carboxylic Food Acids and Salts Category Assessment Plan for EPA's High Production Volume (HPV) Challenge Program¹³ grouped and read across the toxicity endpoints for acetic acid, **ammonium acetate** (AMA), calcium acetate, magnesium acetate, manganese acetate, potassium acetate and sodium acetate to collectively assess their toxicity and reported:

"The chemical structures, physical-chemical properties, environmental fate behavior, and aquatic and mammalian toxicity of these seven compounds are similar. Acetic acid and its salts undergo dissociation in aqueous media into the acetate anion($\text{H}_3\text{C}_2\text{O}_2^-$) and the respective cations (H^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , K^+ , Na^+). The toxicity of each compound is driven by acetate, with the cations in most cases playing a minor role.

The extreme blood ammonia levels reported in the Satpute paper are irreconcilable with the results of other studies. As shown in Table 1 below, other researchers reported blood ammonia levels of about 300-400 $\mu\text{mol/L}$ following repeated dietary doses of ammonium acetate at about 20,000 mg/kg/day, or after repeated i.p. administration of 100 mg/kg/day. However, the Satpute paper states that blood ammonia levels in the range of 100,000 $\mu\text{mol/L}$ were achieved following 28 days of gavage administration of ammonium acetate to rats at 100 mg/kg/day. These are serious discrepancies and call into question the actual dose of ammonium acetate administered in the Satpute *et. al.* work.

Table 1 redacted (copyrighted material)

The Satpute study is technically flawed both in methodology and reporting and should not be considered acceptable, either on its own or with other studies for a weight of evidence assessment of hazard or risk. For example:

1. The purity of the test substance was not defined.
2. Analytical confirmation of the dose concentration was not performed.
3. The pH of the test solution was not determined. It is unknown whether the solution was driven to more toxic ammonia or the less toxic ammonium ion.
4. The number of animals tested was insufficient.
5. Clinical signs of toxicity or neurotoxicity were not recorded and there is no indication that these were examined.
6. The integrity of the GI tract was not assessed.
7. The results of gross necropsy were not reported. It is not possible to verify the integrity of the stomach or intestine.
8. Methodology problems with brain tissue evaluation were apparent. The lack of perfusion and differences in staining of neural tissue slides quality point to the high likelihood of fixation and/or processing artifacts. The photomicrographs of selected neural tissue preparations have insufficient resolutions and quality to validate the reported findings.
9. Body and organ weights were not presented. Only relative organ weights were reported. In the absence of these data it is not possible to determine actual effect

Hope this finds you well,

Nina Wilson

