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## Mechanism for the release of inorganic arsenic from roxarsone, 3-nitro-4-hydroxyphenylarsonic, by cyclic voltammetry at a gold electrode

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### ABSTRACT

Roxarsone, an arsenic-containing poultry-feed additive, is stable in fresh poultry litter but degrades to inorganic arsenic among other products when the litter is composted. Inorganic arsenic is obviously formed via the cleavage of the C-As bond but the mechanism of the cleavage of this bond remains obscure. In this work, cyclic voltammetry was used to study the redox behavior of roxarsone at a gold electrode. An oxidation peak which was absent in blanks and which could not be attributed to redox activities at the nitro and the arsonic acid groups was observed at more positive potentials. The peak appeared even when the anodic scan was initiated at potentials more positive than the peak potential for the first reduction peak indicating that it was due to the oxidation of the bulk analyte, roxarsone. This could be due to the oxidative aromatic fission of roxarsone which will subsequently lead to the cleavage of the C-As bond.

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### KEYWORDS

Roxarsone;  
Oxidative aromatic fission;  
Arsenic.

### INTRODUCTION

Roxarsone (ROX), 3-nitro-4-hydroxyphenylarsonic acid, is an arsenic-containing feed additive that is used extensively in the poultry industry to promote growth by controlling coccidial intestinal parasites. The management of poultry litter (PL) is a challenging environmental problem<sup>[1]</sup> and the practice of amending soils with arsenic-containing PL and the use of uncovered windrows for PL storage could be causing arsenic contamination of surface and ground water. The recent reduction of the maximum contaminant level for arsenic in drinking water from 50 ppb to 10 ppb and the resultant political debate have focused public attention on

this element. The new arsenic contaminant level has a significant impact in the poultry industry and in the management of PL.

Numerous reports have shown that most of the ROX ingested by chickens is excreted unchanged in the manure bedding despite an earlier study by Moody and Williams<sup>[2]</sup> in which 3-amino-4-hydroxyphenylarsonic acid, a metabolite of ROX, was detected in urine of hens fed with ROX. A recent study by Basu et al.<sup>[3]</sup> showed that ROX, like As(III), promote angiogenic phenotype in human epithelial cells. ROX has been shown to be stable in fresh poultry litter but is converted to As(V) and As(III) among other products when the PL is composted and PL collected from a typical

42-day feeding period contains from 30 to 50mg Kg<sup>-1</sup> of total arsenic. Garbarino, et al.<sup>[4]</sup>, examined the aqueous extractability of ROX from PL after one hour at room temperature and reported that the extract contained 70% of the total arsenic. After 48 hours of anaerobic conditions at room temperature, the ROX in this litter had been totally transformed into different arsenic-containing components. The transformation rate followed the usual temperature dependence; however, sterilization rendered the ROX stable for at least 10 days, which suggested the involvement of microorganisms. Two different soil types were amended with PL and examined to determine the effects of soil type on ROX degradation. The aqueous extracts of both a silted and clay pasture soil and a sandy agricultural soil were found to contain mostly arsenate, As (V). In contrast, arsenite, As (III), was the predominate species found in the extract of the fine fraction of bed sediment from a ditch adjacent to the agricultural soil.

It has been suggested that reduction of the nitro group, oxidative aromatic ring fission, and rupture of the C-As bond are the possible environmental reaction mechanisms of ROX. This implies that 3-amino-4-hydroxyphenylarsonic acid, methylarsines, arsenate (AsO<sub>4</sub><sup>3-</sup>), and arsenite (AsO<sub>3</sub><sup>3-</sup>) are possible degradation products. Jackson and Bertsch<sup>[5]</sup> found that 92% of the total arsenic in PL was water-soluble after extraction of 1g of the litter with 10-mL of water for two hours. They identified ROX as the major species (61%) in the extract. Bednar et. al.<sup>[6]</sup>, showed that arsenite, the most toxic form of arsenic, can be photochemically cleaved from ROX at pH 4-8 and then rapidly oxidized to the less toxic arsenate. This suggests photo-induced reductive cleavage of the arsonic acid group from ROX. The degradation rate increased with pH, nitrate concentration and the amount of natural organic matter. They proposed a mechanism in which nitrite, formed by the photolytic reduction of nitrate, replaces the arsonic acid group which leaves as the arsenite. This proposed mechanism, however, can not explain the degradation mechanism of ROX in soil conditions where light can not penetrate. In addition, 2, 4-dinitrophenols have not been identified as some of the products of ROX transformation as this mechanism would suggest.

In a recent study, Cortinas et. al. showed that ROX

is rapidly transformed to 4-hydroxy-3-aminophenylarsonic acid (HAPA) under anaerobic conditions<sup>[7]</sup>. Arsenite was identified as the main product of the degradation of HAPA and 4-aminophenylarsonic acid under methanogenic and sulfate-reducing conditions. They however did not identify the other products and neither did they elucidate the mechanism of the formation of inorganic arsenic. The cleavage of the C-As bond without fission of the aromatic ring should produce 2-aminophenol among other products. Stolz et. al.<sup>[8]</sup> also identified HAPA and inorganic arsenic as the major anaerobic biotransformation products of ROX. Based on electron structure analysis, they suggested that reducing equivalence should go to the nitro group instead of the arsenate group, and that the liberation of inorganic arsenic from the cleavage of the C-As bond from the intact benzene ring was very unlikely. Their electrochemical analysis work suggested that the benzene ring should be oxidized first, leading the release of inorganic arsenic. This is consistent with work by Lowry et. al.<sup>[9]</sup> in which a proposed redox mechanism involving phenylarsine oxide did not involve the cleavage of the C-As bond.

Watson et. al.<sup>[10,11]</sup> proposed an irreversible four-electron reduction mechanism of phenylarsonic acid to phenylarsenoxide. The phenylarsenoxide produced was further reduced in a reversible four electron step to phenylarsine; with arsenobenzene being the final product. The nitro-substituted phenylarsonic acids gave rise to two waves of equal height on a dropping mercury electrode. The less negative wave was attributed to the reduction of the nitro group to the hydroxylamine derivative while the more negative one was due to the reduction of the arsonic acid group. Accordingly, reduction can not possibly lead to the production of inorganic arsenic. The redox behavior of the organic arsenic oxides studied was however not fully explored for the potential reductive cleavage of the C-As bond or the oxidative fission of the aromatic ring; both of which would give rise to inorganic arsenic.

The main objective of this work was therefore to fully explore the redox behavior of ROX using gold Au electrode, evaluating any potential evidence for the mechanism of the formation of inorganic arsenic from ROX.

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### EXPERIMENTAL

Roxarsone, 3-nitro-4-hydroxyphenylarsonic acid (analytical standard), was obtained from Sigma Aldrich (St. Louis, MO) and reagent-grade or higher chemicals (Fisher Scientific, Chicago, IL) were used in all experiments. All solutions were prepared with Milli-Q (millipore, Billerica, MA) deionized distilled water. Glassware used was acid-washed (10% HNO<sub>3</sub>), oven dried (160-170°C) and rinsed with 70% isopropyl alcohol. The gold (Au) working electrode was obtained from BASi Bioanalytical Systems Inc. (West Lafayette, IN, USA). In between experiments, the electrode was polished using the BAS supplied alumina polish and then rinsed with distilled water. The electrode was further sonicated in distilled water using a Sonic Dismembrator (Model 100; Fisher Scientific) for about 10 minutes to ensure complete removal of the alumina particles. An Epsilon Potentiostat coupled to a C3 Cell Stand was used for all the experiments. An Ag/AgCl and a Pt wire (BASi Bioanalytical Systems Inc., West Lafayette, IN, USA) were used as the reference electrode and counter electrode respectively. A model 2100 pH/Temp/mV meter (VWR Scientific Products, West Chester, PA) was used to measure the pH of solutions.

Cyclic voltammetry experiments were designed to study the oxidation-reduction mechanism of ROX using an Au electrode. Blanks were made of deionized water and the acetate buffer pH 4.6. The solutions were purged for about 10 minutes with ultra pure argon gas before and in between experiments. Preliminary 2-cycle experiments had showed reduced peak currents for the first reduction peak on the second cycle; an indication that the analyte was adsorbing on the working electrode. The adsorption effects were alleviated by polishing, rinsing and sonicating the working electrode in between experiments. All the glassware used, including cell vials, was oven dried and rinsed with isopropyl alcohol after washing to avoid potential microbial degradation of ROX during and in between experiments.

### RESULTS AND DISCUSSION

Figure 1 shows the electrochemical behavior of ROX at the gold electrode under the conditions investi-

gated. The magnitudes of the four reduction peaks and the three oxidation peaks increased with scan rate. The first two oxidation peaks, E and F, were much smaller relative to the oxidation peaks; a diagnostic feature for a coupled chemical reaction involving product(s) of the reduction reactions. Peak G was independent of the reduction products and their fate indicating that it was due to the oxidation of the bulk analyte. As shown in Figure 2, the oxidation-reduction peak currents generated at the gold electrode were directly proportional to the square root of scan rate. This is consistent with the Randles-Sevcik equation for diffusion-controlled currents:

$$i_p = (2.69 \times 10^5) n^{2/3} A D_0^{1/2} v^{1/2} C_0^* \quad (1)$$

where:  $i_p$  is current in amperes,  $n$  is number of electrons,  $A$  is electrode surface area in cm<sup>2</sup>,  $D_0$  is diffusion coefficient in cm<sup>2</sup>/sec,  $v$  is scan rate in volts/sec, and  $C_0^*$  is bulky concentration in moles/cm<sup>3</sup>.

The redox system also obeyed the Laviron equation for irreversible adsorbed systems as shown in figure 3:

$$E_p = E^\circ + RT/(\alpha nF) [\ln[(RTk_s)/(\alpha nF)] - \ln v] \quad (2)$$

Where:  $k_s$  is the charge transfer rate constant,  $\alpha$  is electron transfer rate constant,  $F$  is the faraday constant,  $R$  is the Universal gas constant,  $T$  is the Kelvin temperature and  $E^\circ$  is the formal reduction potential. This implies that ROX diffused to the electrode surface and got adsorbed before being oxidized or reduced in a mechanism consistent with that proposed by Forryan et. al. for 4-nitrophenol at a gold electrode<sup>[12]</sup>. Electrochemical reversibility is a matter of degree and most redox systems tend to be quasi-reversible as scan rate is increased, with reduction peaks shifting to more negative potentials and oxidation peaks shifting to more negative potentials. This was the case with the ROX redox system in this study as shown in figure 4. Adsorption of analyte on the working electrode was evident from preliminary multi-cycle experiments in which the first reduction peak decreased in size on the second cycle. The adsorption effects on successive scans were alleviated by polishing, rinsing and sonicating the working electrode in between experiments. A similar observation was reported by Hutton et al. for nitrophenols on a bismuth film electrode<sup>[13]</sup>.

According to literature, reducing equivalents should

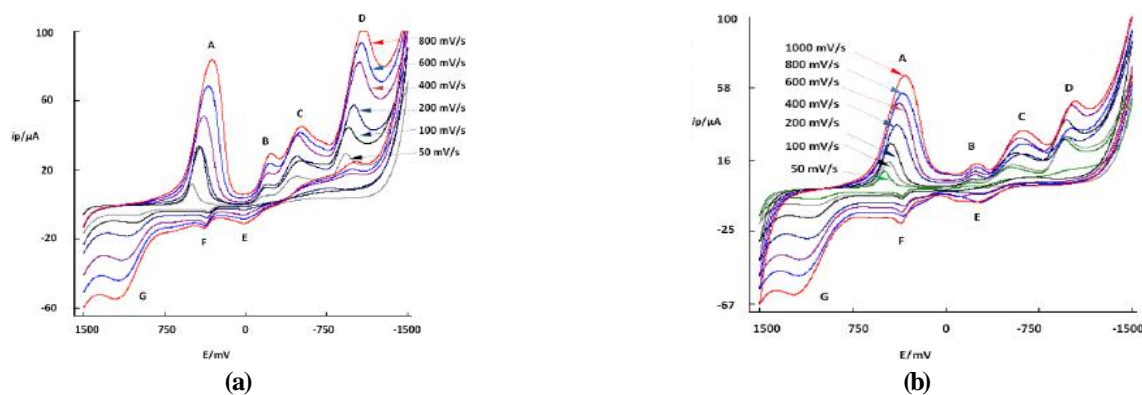


Figure 1 : (a) Cathodic-anodic scans and (b) anodic-cathodic scans for 1.2 mM roxarsone in pH 4.6 acetate buffer

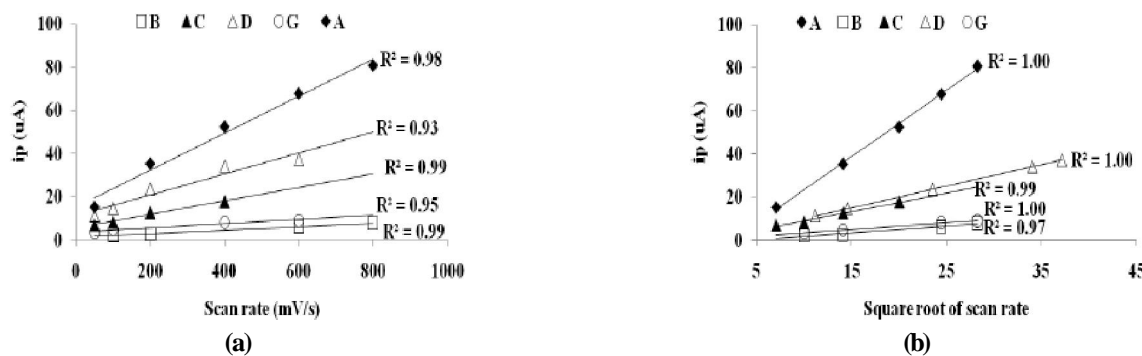


Figure 2 : Effect of scan rate on peak current for a 1.2 mM solution of roxarsone in acetate buffer pH 4.46: (a) peak current versus scan rate; (b) peak current versus square root of scan rate

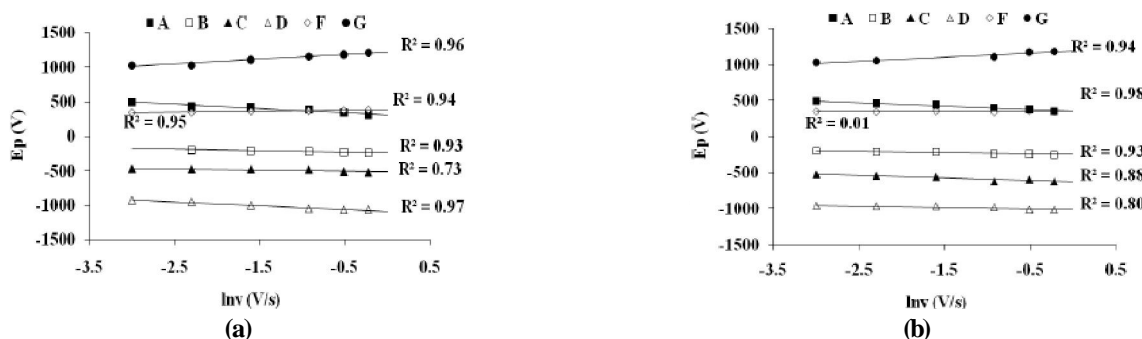


Figure 3 : Effect of scan rate on peak potential for a 1.2 mM solution of roxarsone in pH 4.46 acetate buffer: (a) cathodic-anodic scans and (b) anodic-cathodic scans

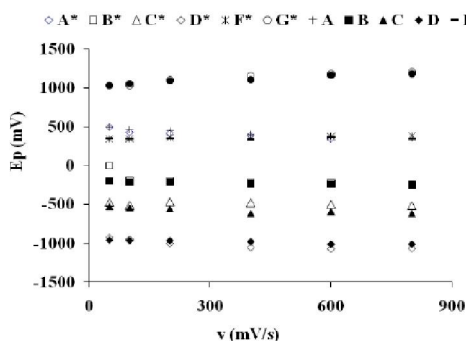


Figure 4 : Effect of scan rate on peak potential for a 1.2 mM solution of roxarsone in pH 4.46 acetate buffer with cathodic-anodic scans (open symbols) and anodic-cathodic scans (solid symbols) on the same axis

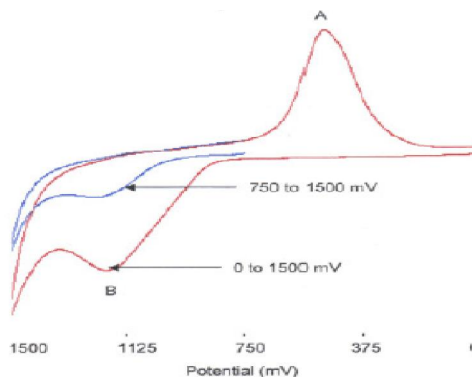
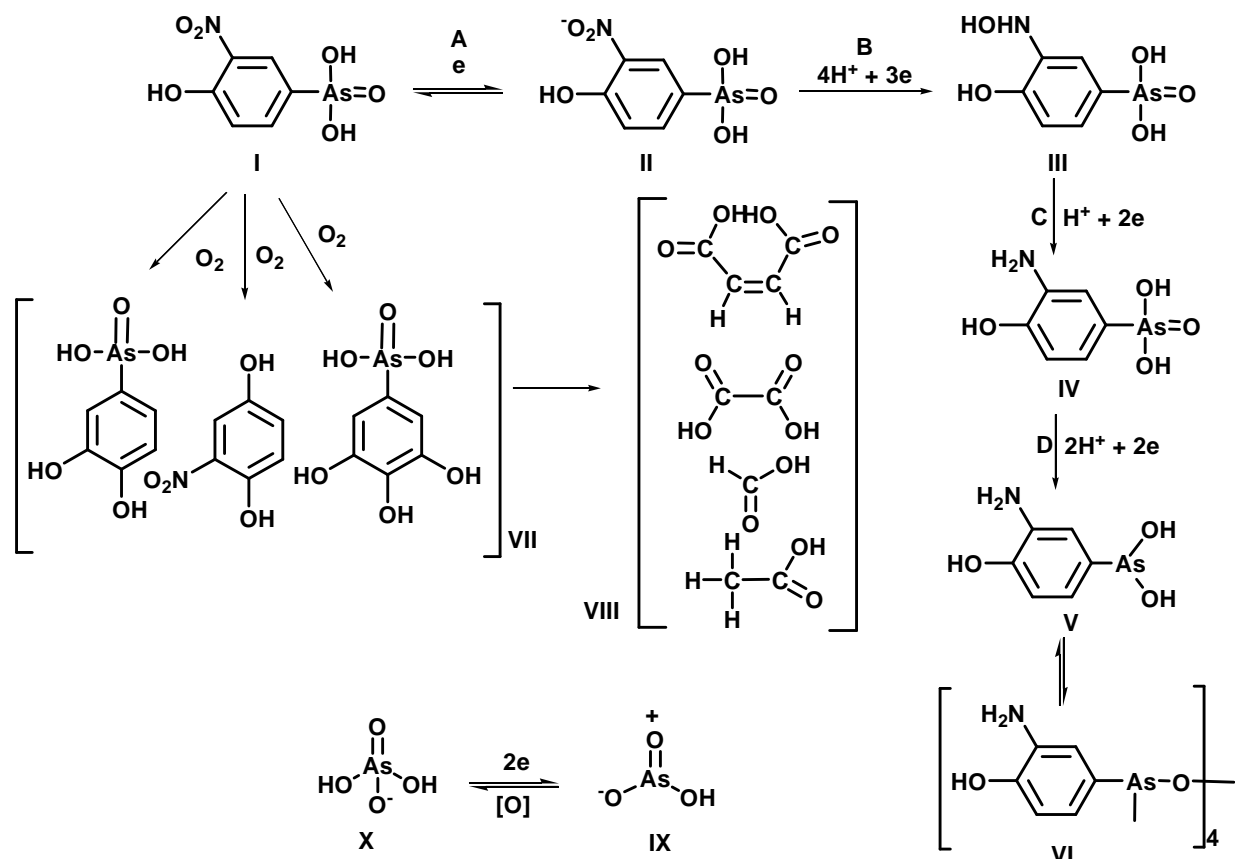


Figure 5 : Anodic-cathodic scans for a 1.2 mM solution of roxarsone in pH 4.46 acetate buffer. Red: 0 mV to 1500 mV scan range; Blue: 750 mV to 1500 mV scan range

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**Figure 6 :** The proposed redox reaction mechanism involving ROX at a gold electrode. I: ROX (3-nitro-4-hydroxyphenylarsonic acid); II: 3-Nitroso-4-hydroxyphenylarsonic acid; III: 4-Hydroxy-3-hydroxylaminephenylarsonic acid; IV: 4-Hydroxy-3-aminophenylarsonic acid; V: 4-Hydroxy-3-aminophenylarsenoxide; VI: Oligomer; VII: Oxidation intermediates/precursors of ROX; VIII: Products of oxidative aromatic fission; IX: Arsenite (product of oxidative aromatic fission) and X: Arsenate (product of oxidative aromatic fission). A, B, C, & D corresponds to electrochemical processes responsible for peaks A, B, C, D on the gold electrode

go to the nitro group first<sup>[8]</sup> so peak A is probably a result of a one-electron reduction of ROX to the nitroso derivative while B is a three-electron reduction of this product to 4-Hydroxy-3-hydroxylamine phenylarsonic acid. Peak C is likely due to the reduction of the hydroxylamine derivative to 4-Hydroxy-3-aminophenylarsonic acid. Peak D could be due to the reduction of the arsonic acid group of 4-Hydroxy-3-aminophenylarsonic acid to 4-Hydroxy-3-aminophenylarsenoxide which is expected to then form an oligomer. Phenylarsenoxides have been shown to form oligomers<sup>[10,11]</sup>.

This gives an electrochemical-electrochemical-electrochemical-chemical (EEEC) reaction mechanism. Similar redox behaviors were reported for nitro- and arsonic acid substituted aromatic compounds. As a result of the proposed chemical step (formation of the oligomer), the return peaks E and F, corresponding to the oxidation of 4-Hydroxy-3-aminophenylarsenoxide

and 4-Hydroxy-3-hydroxylaminephenylarsenoxide respectively, were very small and virtually absent at slow scan rates. Peak G was the most important peak for this study because it has not been reported before for ROX and similar compounds. The peak is probably due to the oxidation of the benzene ring which would eventually lead to the cleavage of the C-As bond. Arsenic (V) and As(III) have been shown to be some of the major degradation products of ROX in the natural environment<sup>[6-10]</sup> and our work suggest that they could be formed via oxidative aromatic fission. As shown in Figure 4, peak G appeared even when the anodic scan was initiated at a potential more positive than the potential at which peak A, the first reduction peak of ROX, was appearing, suggesting that the peak was due to the oxidation of the bulky analyte, ROX. However, the peak (G) became larger when the anodic scan was initiated at a potential just negative of the peak potential for peak



A. This could have been due to an overlap between the oxidation of ROX and the oxidation of the nitroso-derivative to ROX. The peak was absent in the blanks. The proposed scheme of reactions is shown in figure 6.

The proposed aromatic ring fission is hypothesized to be preceded by hydroxylation of the unsubstituted aromatic positions and denitro/deamination-hydroxylation reactions. Cleavage of the C-As bond which is hypothesized to occur after ring fission could also occur prior to ring fission via a dearsonification-hydroxylation mechanism. Other products for the proposed oxidative aromatic fission of ROX which are not shown in figure 6 include  $\text{CO}_2$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ . The As(III) produced is expected to be methylated and transformed via the Challenger mechanism<sup>[14]</sup>. Arsenoalkyl acids intermediates can be converted to alkylarsines under anaerobic conditions but will be rapidly oxidized to arsenate upon exposure to aerobic conditions.

The transformation of ROX into toxic arsenic species makes the management of PL a real environmental challenge and the potential contamination of surface and groundwater cannot be overemphasized. Fish and other edible aquatic organisms will bioaccumulate the As compounds and pass them on to human beings through the food chain. Volatile arsines, which are some of the transformation products of ROX, can be accessed by humans through inhalation. Eating chicken exposed to ROX could be one of the direct sources of ROX for humans. There are some communities that consider the chicken gut a delicacy, making themselves more vulnerable to the effects of ROX and its transformation products.

## CONCLUSION

In this work, experimental evidence for possible oxidative aromatic fission of roxarsone which could explain why inorganic arsenic is one of the major degradation products of roxarsone has been shown for the first time. More work is being done in my laboratory to fully elucidate the electrochemical mechanism of the oxidative aromatic fission of ROX and the subsequent release of inorganic arsenic in composted PL. Considering the potential contamination of freshwater sources with ROX and its degradation products, the potential toxicity of ROX and the devastating health effects of

As, it may be prudent to treat PL for As before applying it onto land as manure or better still find safer alternatives to ROX.

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