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CORRESPONDENCE/REBUTTAL

Rebuttal to a Comment on New Reagent for Trace Determination of Protein-Bound Metabolites of Nitrofurans in Shrimp Using Liquid Chromatography with Diode Array Detector

Recent concerns were expressed on the validity of using semicarbazide as the marker of nitrofurazone abuse. In support of these concerns, it was pointed out that crayfish samples were known to contain semicarbazide (SEM) and that semicarbazide can be formed from hydroxyurea under various pH conditions. Also, on the basis of the apparent similarity between shrimp and crayfish, which are both decapod crustaceans, it was suggested that shrimp may likely contain semicarbazide, leading to false-positive results. Additionally, it was pointed out that no specific form of metabolites of nitrofurans are given under European Union (EU) legislation. Finally, it was also suggested that 5-nitro-2-furaldehyde may be used in cases of food samples in which nitrofurazone depletes rapidly.

It is acknowledged that there could be other sources of SEM in tissue samples which are not related to the illegal use of nitrofurazone. However, in the paper reporting that wild crayfish samples contain SEM (*1*), shrimp was in fact used as a reference material for a negative control sample and for the spiking experiment. This indicates that SEM content was measured in shrimp and found to be a nonissue.

Additionally, according to a proficiency study for the determination of nitrofuran metabolites (AHD, AOZ, AMOZ, and SEM) in shrimps reported by Hurtaud-Pessel et al. (*2*), there were no false-positive results on SEM found among the 20 collaborative laboratories. However, false-positive results on AMOZ and AOZ were found by two laboratories.

We concur that there are no characteristic compounds for nitrofuran metabolites that are mentioned in the EU legislation (*3*). Therefore, in our paper on page 1753, the first line on the left column is to be modified as "no characteristic compound for nitrofurazone metabolites is given under EU legislation and recent development methods for nitrofuran analysis still utilize SEM as marker residue for nitrofurazone abuse".

With regard to the suggestion for the use of 5-nitro-2-furaldehyde as a marker for nitrofurazone abuse, it is likely acceptable if one is interested in the analysis of intact nitrofurazone only. However, if one is interested in the analysis of protein-bound metabolites of nitrofurazone, 5-nitro-2-furaldehyde is not appropriate as in vivo the nitro group of the nitrofuran part is reduced, whereas the side chain, SEM, remains unaltered (*4*). Additionally, it was recently suggested that SEM forms part of a nitrofurazone metabolite conjugated to glutathione (*5*).

Finally, the method we developed is a screening method. This indicates that a suspected-noncompliant result obtained from this method shall be confirmed by a confirmatory method (*6*).

LITERATURE CITED

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