MARINE MAMMAL SCIENCE, 23(4): 991–992 (October 2007)

DOI: 10.1111/j.1748-7692.2007.00157.x

Reply to Wetzel *et al.*'s Comment: "Identification of Fatty Acids by Picolinyl Ester Derivatives"

The comment of Wetzel *et al*. (2007) on our review (Budge *et al*. 2006) raises several important points about the analysis of fatty acids (FA) with gas chromatography-mass spectrometry (GC-MS), and we appreciate the opportunity to clarify our position.

We certainly did not intend to dissuade anyone from using GC-MS. We agree that it is a valuable analytical tool in lipid analysis, particularly for structure determination, whether applied to FA methyl esters (*e.g.*, Dayhuff and Wells 2005) or nitrogen-containing FA derivatives (*e.g.*, Christie 1998, Wetzel and Reynolds 2004). Our aim was to emphasize that GC-MS, although very useful, is not necessary for routine FA analysis and to underscore that GC-flame ionization detection (FID) of methyl esters using a polar column is very reliable. Our concern arose because recent presentations and papers (*e.g.*, Wetzel and Reynolds 2004) have stated that the long-standing and most commonly used technique for FA analysis, GC-FID of FA methyl esters, cannot "effectively differentiate double bond isomers or branched components" and "would only allow tentative identification of [58%] of those FA in [a] sample." These are misleading statements, especially because they were based on the GC-FID analysis of FA methyl esters using a nonpolar column, which is well known to be unable to resolve most marine isomers. As Wetzel *et al*. (2007) readily acknowledged, the use of GC-MS analysis of picolinyl esters is extremely costly in both time and instrumentation. Thus, our comments were made to ensure that no one was discouraged from conducting FA analyses because of cost or lack of access to GC-MS. We were simply trying to dispel the notion that accurate and precise FA analysis is only possible with the GC-MS analysis of picolinyl esters.

We also did not suggest that identification of fatty acids by picolinyl ester derivatives was inappropriate, as asserted in Wetzel *et al*. (2007). Instead, we believe and did clearly state that "nitrogen derivatives, such as picolinyl esters, can be very useful in originally identifying unknown FA structures" but that it is "not necessary to employ these, or similar FA derivatives, each time a sample is analyzed for FA content." Indeed, we are presently using one such derivative, 4,4-dimethyloxazoline (DMOX) coupled with GC-MS, to identify unusual FA structures in a variety of marine samples. However, once the structure of the FA in question has been determined, we then return to analysis of the methyl ester derivatives by GC- FID using a polar capillary column to quantify the FA composition of the sample.

As we stated in our review, our real concern with the use of picolinyl esters and other derivatives involves their use in the quantitative determination of FA. Although Wetzel *et al*. (2007) provide an example of separation of some monounsaturated FA isomers present in low amounts (Nechev *et al*. 2004), we still maintain that this is not an easily accomplished task and that the use of picolinyl derivatives will usually lead to co-elution of those isomers. It is very difficult to accurately quantify any compound if co-elution has occurred. We agree that other nitrogen-containing derivatives exist with better chromatographic properties than picolinyl esters. Yet, even with improved resolution of DMOX derivatives, for example, methyl esters remain the derivative that provides the best resolution with GC, whether by GC-MS or GC-FID. Thus, the issue has nothing to do with the detection system; both MS and FID are appropriate. It is the GC resolution that remains the difficulty in quantification of derivatives

other than methyl esters. Therefore, given that all structures have been accurately determined, we stand by our assertion that analysis of FA methyl esters by GC is the most reliable and accessible method for routine quantification of FA composition.

LITERATURE CITED

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S. M. BUDGE, Department of Process Engineering and Applied Science, Dalhousie University, Halifax B3J 2X4, Nova Scotia, Canada; e-mail: suzanne.budge@dal.ca; **S. J. IVERSON**, Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada and **H. N. KOOPMAN**, Biological Sciences, University of North Carolina at Wilmington, 601 South College Road, Wilmington, North Carolina 28403, U.S.A. Received 8 June 2007. Accepted 20 June 2007.