

Phthalates and the Overestimation of Docosanoic Acid in Seal Lipids

Sir:

In a recent paper, we (Durnford and Shahidi) (1) reported the FA composition of a variety of tissues of phocid seals. We have since examined our results further as well as those from previous work (2,3) and also repeated the storage of tissue samples and of samples for FA analysis in all-glass containers instead of plastic vessels. In doing so, we did not detect the levels of docosanoic acid (22:0) that we had earlier reported (1). Ackman, who has considerable experience with possible contamination arising from phthalate plasticizers, suggested that overestimation of 22:0 may have been due to the presence of a contaminating dibasic phthalate ester from plastic laboratory vessels (Ackman, R., personal communication). As discussed in detail previously (4), it is known that, during methylation or transesterification of FA, contaminating plasticizers such as isobutyl, butyl, 2-ethylhexyl, or octyl double alcohol phthalates may be converted partially to a monomethyl ester, retaining one of the original alcohols from this transesterification. Although di-(2-ethylhexyl)phthalate itself emerges after the 22:6n-3 methyl ester position (3,4) on a polyglycol-based GLC column, and fully methylated phthalic acid is found in the vicinity of the 17:0 FAME, in the case of dioctyl phthalate the mixed ester phthalate will occupy an intermediate position, usually between the methyl esters of 20:4n-6 and 22:1 on the GLC chart. Welz *et al.* (5), for example, show what appears to be the original and mixed alcohol phthalates as two very large unidentified peaks in an analysis of FA of human plasma lipids. Clinical plastic equipment is an obvious potential source of such plasticizers. Fortunately for the modern analytical chemist, the phthalate ring still yields a convenient and distinctive mass spectrum (4).

When work was done in glass containers, blubber and muscle lipids were the only tissues for which differences were significant for 22:0. Values in muscle tissues for gray, harp, and hooded seals were all at $1.81 \pm 0.32\%$. For blubber, the corresponding values for the same species were less than 0.3%. For brain, kidney, heart, and lung tissues, the existing differences from those reported earlier were generally within the range of SD values reported. Other research reports support our findings (6,7). Nonetheless, the conclusions reached in the previous report (1) are not affected by the observed overestimations, but would afford proportionally higher

amounts of nutritionally important n-3 FA in the lipids of concern.

For lipid research ending in FAME assays, all solvents, samples, and extracts should preferably be handled in glass. As exemplified by Welz *et al.* (5), clinicians favor soft bags and tubing, rich sources of phthalates.

REFERENCES

1. Durnford, E., and F. Shahidi, Comparison of FA Compositions of Selected Tissues of Phocid Seals of Eastern Canada Using One-Way and Multivariate Techniques, *J. Am. Oil Chem. Soc.* 79:1095–1102 (2002).
2. Ackman, R.G., and F. Lamothe, Marine Mammals, in *Marine Biogenic Lipids, Fats and Oils*, edited by R.G. Ackman, CRC Press, Boca Raton, 1989, Vol. 2, pp. 179–381.
3. Wanasundara, U.N., and F. Shahidi, Stabilization of Seal Blubber and Menhaden Oils with Green Tea Catechins, *J. Am. Oil Chem. Soc.* 73:1183–1190 (1996).
4. Shantha, N.C., and R.G. Ackman, Behaviour of a Common Phthalate Plasticizer (dioctyl phthalate) During the Alkali and/or Acid-Catalysed Step in an AOCS Method for the Preparation of Methyl Esters, *J. Chromatogr.* 587:263–267 (1991).
5. Welz, W., W. Sattler, H.-J. Leis, and E. Malle, Rapid Analysis of Non-esterified Fatty Acids as Methyl Esters from Different Biological Specimens by Gas Chromatography After One-Step Esterification, *J. Chromatogr.* 526:319–329 (1990).
6. Fredheim, B., S. Holen, K.I. Ugland, and O. Grahl-Nielsen, Fatty Acid Composition in Blubber, Heart and Brain from Phocid Seals, in *Whales, Seals, Fish and Man*, edited by A.S. Blix, L. Walløe, and Ø. Ulltungen, Elsevier, Amsterdam, 1995, pp. 153–168.
7. Henderson, R.J., N. Kalogeropoulos, and M.N. Alexis, The Lipid Composition of Selected Tissues from a Mediterranean Monk Seal, *Monachus monachus*, *Lipids* 29:577–582 (1994).

E. Durnford^{a,b}, F. Shahidi^{a,*}, and R.G. Ackman^c

^aDepartment of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada,

^bSchool of Fisheries,

Institute of Fisheries and Marine Technology of Memorial University of Newfoundland,

St. John's, Newfoundland A1C 5R3 Canada, and

^cCanadian Institute of Fisheries Technology, Dalhousie University, Sexton Campus, D-401, Halifax, Nova Scotia, B3J 2X4 Canada

[Received February 24, 2003; accepted February 27, 2003]

Paper no. J10576 in *JAACS* 80, 405 (April 2003)

*To whom correspondence should be addressed.

E-mail: fshahidi@mun.ca