

Letter to the Editor

Correspondence re: Letter to the Editor by B. K. Tang and W. Kalow on
CYP1A2 Phenotyping Using Caffeine. *Cancer Epidemiol.
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The following comments pertain exclusively to a Letter to the Editor by B. K. Tang and W. Kalow (1) that concludes that the "Butler methods" developed by our laboratory (2) for determining CYP1A2 activity are inadequate and that adoption of these methods "in cancer research would lead to results that are not much better than half-truths." Because our method, which measures the urinary molar ratio of (17X + 17U)/137X, and that of Tang and Kalow (3), which uses the urinary ratio of (AAMU + 1X + 1U)/17U, are both being used in a variety of cancer epidemiology and biomarker studies, we again wish to comment on the validity of our original method.

The basis for the conclusions of Tang and Kalow is derived from previous work by these authors (3) in which they reported that caffeine (137X) excretion is urine flow-dependent and that our ratio of (17X + 17U)/137X reflects only a polymorphism in renal clearance of caffeine. We have previously stated (4) that, although caffeine clearance is indeed dependent on urine flow, we could find no evidence that caffeine urinary concentrations are urine flow-dependent under our experimental conditions and methods of analysis. Furthermore, our results agree with those of Birkett and Miners (5), who have also reported that urinary caffeine concentrations are independent of urine flow rate. In a recent study,² in which we found comparable trimodal distributions for both the (17X + 17U)/137X ratio and the caffeine breath test, we noted a urinary constituent that often co-migrates with caffeine on a variety of HPLC³ columns. Moreover, its excretion was highly dependent on urine flow rate. This constituent is typically present in 20–40% of urine samples that we have examined and seems to be of dietary origin. Accordingly, our selection of HPLC columns for use in caffeine urinary metabolite determinations has required the use of computerized photodiode-array detection systems that assure peak purities by comparisons with spectral libraries of authentic standards. Generally, we have found a similar proportion (1:1:1) of columns (even from the same manufac-

turer) in which this unknown constituent co-elutes with caffeine, is partially separated, or suitably elutes about 1 min before the caffeine peak. Although it is difficult to conclude whether this explains the difference between our results and those of Tang and Kalow, we note that they do not appear to use diode-array UV detection and spectral matching to assure analytical separation of this constituent from caffeine. This may also be the case in a recent study by Notarianni *et al.* (6), who expressed similar concerns about the accuracy of caffeine as a probe for CYP1A2 activity and, like Tang and Kalow in the Letter to the Editor, advocate the use of variable urine collection times.

As we have previously stated (2), our experience is that reproducibility for our method is always optimal at 4–5 h. The smallest peak on the HPLC elution profile is always caffeine, and a 4–5 h urine sample usually contains the highest concentrations of caffeine because that is the time at which blood caffeine concentrations are highest. Urines collected at 3–4, 5–6, or 7–8 h have lower caffeine concentrations, and such measurements are inherently less accurate. Recently, Denaro *et al.* (7) examined the use of 24-h or spot urine collections and concluded that the ratio of (AAMU + 1X + 1U)/17U is a valid measure of caffeine clearance and that the use of other ratios is questionable. We agree with this assessment for 24-h or spot urine samples and again emphasize the need for 4–5 h urine collections for measuring (17X + 17U)/137X. Moreover, this study (7) and others (3, 6) regard caffeine clearance as the gold standard for reflecting CYP1A2 levels in liver. We do not believe that there is any experimental support for this assumption and tend to regard the (3-¹³C)caffeine breath test (8) as the most direct measure of CYP1A2-catalyzed caffeine 3-demethylation in the liver.

Tang and Kalow further state in their Letter to the Editor "that all other methods using caffeine for CYP1A2 determination lead to normal distribution curves," whereas we and our collaborators have reported bimodal or trimodal distributions. However, Fuhr and Rost (9), who used 17X/137X ratios in plasma and saliva, found bimodal distributions for CYP1A2 activity in both smokers and nonsmokers. These findings are also in accordance with family studies and *in vitro* metabolism data that have consistently shown a heritable bimodal/trimodal distribution for CYP1A2 activity. Yet these data do not necessarily imply that there must be a functional variant in the CYP1A2 gene (10), since a polymorphic phenotype can arise from mutations in regulatory genes or can be due to individual differences in long-term exposures to agents that alter hepatic CYP1A2 levels.

Most studies to date have used probit plots to depict CYP1A2 phenotypic distributions. However, we wish to stress that probit plots are simply graphical methods that are useful to assign cutpoints and are not statistical tests (11). Perhaps these controversies can be fully resolved by using more accurate

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² F. F. Kadlubar, E. P. Horn, G. H. Lambert, M. A. Tucker, N. E. Caporaso, manuscript in preparation.

³ The abbreviation used is: HPLC, high-performance liquid chromatography.

statistical approaches, by conducting additional family and nutritional epidemiological studies, and by sequencing genes that control CYP1A2 expression or inducibility.

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