
LETTER TO THE EDITOR

Delayed excretion of urinary metabolites after styrene exposure: Fact or artifact?

Aromatic hydrocarbons with side chains are mainly metabolized into aromatic acids, eg, toluene into hippuric acid, xylene into methylhippuric acid, and styrene into mandelic acid (MA) and phenylglyoxylic acid (PGA). In the case of toluene and xylene the urinary excretion of the metabolites has been shown to be fast, and their maximum excretion rate has been found to occur at the end of workday exposure (6, 22). For styrene some authors (23, 24) have emphasized, on the basis of their findings, that the maximum excretion rate of the urinary styrene metabolites, mainly MA, has appeared to be strongly delayed after the end of exposure. Such a finding would cast doubt on the validity of the traditional end-of-shift sampling time which is very frequently used for biological monitoring.

The purpose of this letter is not to discuss the best sampling time for the biological monitoring of workers exposed to styrene — this has been done elsewhere (14) — but rather to comment on some of the points which have led the aforementioned authors to believe in a delayed excretion of the metabolites. In addition we present some arguments and facts in order to examine the extent to which such a delay can be real or must be considered an artifact.

A review of the literature on this subject reveals nine papers with information or figures attesting to the presence or absence of a delayed excretion of the urinary metabolites of styrene. Four of these papers (1, 17, 18, 23) mention a delay of up to 8 h. The first three of these are based

on colorimetric methods which are known nowadays to be nonspecific and prone to systematic blank errors. The only study that has been made with the more specific gas chromatographic method (23) draws its conclusions from a statistically unsatisfactory group of two exposed workers. In addition the analytical results are not fully consistent with current knowledge on the pharmacokinetics of styrene, and they raise some methodological questions (considered later). Another paper (24) uses indirect argumentation for a delayed urinary MA excretion of 8 h by referring to fig 2 of the publication of Bardodej & Bardodejova (2). However, upon examining that paper, one finds that there has been a misinterpretation of the time scale of fig 2 on page 208: the time after the beginning of the exposure was confused with the time after the end of exposure. However, Bardodej & Bardodejova are very clear, saying explicitly on page 207 that "In inhalation experiments the elimination maximum of mandelic acid was observed at the end of exposure." Therefore much of the discussion made by Wolf et al cannot be valid (eg, fig 4, page 328). None of the other authors who have used gas chromatographic methods for analysis (5, 8, 11, 15) have reported any evidence for a significantly delayed urinary elimination of MA.

As far as PGA is concerned, the situation is much more complicated because delicate problems are involved in PGA analysis. For the gas chromatographic methods, mainly two techniques have been used for PGA derivatization: diazomethane methylation and trimethyl silyl (TMS) derivatization. The use of diazomethane for PGA analysis has been extensively studied and discussed in our first paper on styrene

(3). The conclusions drawn there have subsequently been confirmed by other publications (12, 20).

We had demonstrated that the direct methylation of PGA with an excess of diazomethane leads to a varying proportion of secondary methylation products, and therefore using this reaction for analytical work requiring high accuracy and precision is precluded. Indeed, if the diazomethane derivatization product for PGA described by Schaller et al (21) is compared to the one obtained by Buchet et al (4), who originally proposed this method for PGA analysis, it becomes evident that these authors have obtained different derivatives although they used the same method. Schaller's derivative is very likely the methyl ester of PGA, and Buchet's derivative must be a secondary derivative with a higher molecular weight. This failure to reproduce previous results shows clearly the difficulties involved in the diazomethane procedure, which can only be overcome by special derivatization techniques (3, 12).

TMS derivatization has proved to yield an unstable derivative with PGA. Nonreproducible low yields of the corresponding derivative (13) or no derivative at all (10) have been obtained. This finding is consistent with the results of Wilson et al (23) which have already been mentioned. In that work the PGA levels are generally lower than expected, a finding in contrast to current knowledge on the metabolism and kinetics of styrene (the ratio MA/PGA normally changes from 3 at the end of the workshift to below 1 the next morning).

Moreover, the instability of PGA upon standing in urine makes its reliable quantitation rather difficult and requires a transformation of PGA to a stable form prior to gas chromatographic analysis. We have therefore developed a procedure for PGA which involves reduction to MA prior to derivatization and gas chromatographic analysis (13, 15).

As far as elimination kinetics are concerned, we have made experimental human exposures using the reductive PGA determination method and have observed an elimination delay, with respect to the end of the exposure, of about 2 h for the

maximum excretion rate. Since, in terms of metabolism, MA is the precursor of PGA and there is no evidence of a difference in renal excretion rates, the delay for the maximum excretion of MA must therefore be definitely shorter than 2 h. In addition a simplified theoretical approach by mathematical simulation (7) confirms these results.

A short delay in the excretion of toluene and xylene in breath has been reported after skin resorption (9, 16). A potential contact of the hands with liquid styrene at the end of the workday, as can occur in industrial situations, could hypothetically lead to a small delay in the excretion of metabolites. Special conditions such as high exposure shortly before the end of the workday could also explain some delay. On the whole, however, the fact that the metabolism of styrene is very fast and that urinary excretion starts within 1 h after the beginning of exposure (19), as well as the points already discussed, strongly support the conclusion that the delayed excretion of MA reported in the literature is unlikely to be real, and it should hence be considered an artifact.

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