A Reply to "Problems Involved with Developing a Suitable Model for Evaluating Exposure to Bis(2-ethylhexyl) Phthalate from Medical Devices"

In February of this year, we reported a study in rats which indicated that chronic oral administration of the phthalate plasticizer, bis(2-ethylhexyl) phthalate (I), can result in induction of antipyrine metabolism[1]. Based on this observation, we hypothesized that the apparent stimulation of antipyrine clearance reported in earlier clinical studies in chronic renal failure patients could be the result of inadvertent exposure to I during hemodialysis treatment. In a subsequent commentary[2], which appeared in the June issue of this journal, Lawrence and Autian questioned the validity of our hypothesis on the grounds that the metabolism of I may differ between parenteral and peroral routes of administration. They cited various literature reports[3-5] which showed that I can be readily hydrolyzed to form mono(2-ethylhexyl) phthalate (II) in various tissues and biological fluids. The rates of hydrolysis were notably rapid in intestinal tissues and the gut contents[5]. Authors of several of these cited articles had stated that orally administered I *would probably* be absorbed from the GI tract primarily as the monoester. Apparently based on these statements, Lawrence and Autian drew the conclusion that II is formed much more extensively (or even exclusively) after oral administration as compared with after parenteral administration. Since there are data indicating differences in biological activities between I and II[6-9], Lawrence and Autian suggested that parenteral studies with I (which presumably better mimic the introduction of I into patients during hemodialysis) should be performed before our results on antipyrine disposition can be accepted as being valid.

There is no disagreement that hydrolysis of I to II can occur in the gut and/or liver after oral administration. However, we would caution that all of the literature articles cited by Lawrence and Autian were reports of *in vitro* studies on the hydrolysis of I by blood and tissue lipases. It is not possible to draw definite conclusions in regards to the *extent* of oral "first-pass" mono-deesterification of I in the various animal species studied. In fact at the time of our study (*ca.* 1981), detailed *in vivo* pharmacokinetic studies of I and its derived monoester were not available. Furthermore, it was not possible to rule out the existence of a significant degree of mono-deesterification following systemic introduction of I (such that the *overall* extent of formation of II would be comparable between enteral and parenteral administration). For example, it is conceivable that the diester can be excreted in the bile and be subjected to hydrolysis during transit through the intestinal tract. Also, extensive systemic hydrolysis of I certainly can occur.

We were aware of the literature on *in vitro* enzymatic hydrolysis of I (and the lack of quantitative information) at the outset of our study. More importantly, it was recognized that further *in vivo* studies were needed to address the unanswered metabolic and pharmacokinetic questions. We have, over the last 2 years, undertaken a series of studies in rats and in hemodialysis patients which provided much greater insights into the relative pharmacokinetic and pharmacological importance of I and its derived monoester. The results of these studies are currently under review for publication. We believe a brief summary of the relevant data will clarify the question concerning the factor of route of administration in the effect of I on antipyrine metabolism and will illustrate the complexities of the issue.

Our first follow-up study was aimed at elucidating the pharmacokinetics of I and derived II following a single intra-arterial (100 mg/kg), intraperitoneal (4 g/kg), or peroral dose (2 g/kg) of the diester. The key finding from this acute dosing study was a striking difference in the relative concentrations of the monoester metabolite to the parent diester between the parenteral and peroral routes of administration. The area under the plasma concentration–time curve (AUC) ratios of II to I were 0.050 ± 0.024, 0.35 ± 0.27, and 6.90 ± 1.75, respectively. A similar difference in AUC ratios between intraperitoneal and oral routes was found during repetitive administration of I. Using the pharmacokinetic methodologies proposed by Pang and Kwan[10] together with AUC data obtained after direct administration of II, we estimated that ~80% of an oral dose of I was hydrolyzed to II during first passage through the GI tract whereas <2% of the intra-arterial or intraperitoneal dose was mono-deesterified in the liver and other systemic tissues.

Our next step was to compare the effect of I on antipyrine clearance following repetitive intraperitoneal and oral administrations of I in the rat. The observed route dependency in the deesterification of I did indeed result in a difference in the effect of the phthalate plasticizer on antipyrine metabolism, although the results were much more complex than were anticipated. The complicating factor (which was alluded to in our earlier publication) was the elapsed time between the last dose of I and the administration of the antipyrine test. Consistent with our earlier study, a marked degree of induction in antipyrine clearance (an approximately twofold increase) was observed at both 4 and 48 h after the last oral dose of the phthalate ester. On the other hand, the effect after intraperitoneal treatment with I was variable depending on the time at which the antipyrine test was performed. At 48 h postadministration of I, a small degree of *inductive* effect (an ~25% increase in antipyrine clearance) was observed. At the earlier time point (*i.e.*, 4 h), either *no effect* or an apparently *inhibitory* effect was observed. The most likely explanation, which would take into account both the route- and time-dependency in the metabolic effects, is that induction is largely associated with the monoester and that the diester exerts an opposing effect, *i.e.*, inhibition of antipyrine metabolism. Indeed, we were able to demonstrate pronounced inhibition of antipyrine clearance after an acute dose of the diester given either intraperitoneally or orally.

Although our new findings in the rat seem to support the contention of Lawrence and Autian that the study on antipyrine metabolism should have been conducted after parenteral pretreatment of I, we have further data in hemodialysis patients which suggest that neither oral nor intraperitoneal administration of I in the rat exactly mimic the situation during clinical exposure. Studies in a group of renal failure patients on maintenance hemodialysis showed that the circulating concentrations of derived II were comparable to the parent diester during a 4-h dialysis session (AUC ratio of 2.42 ± 0.66). This suggests that mono-deesterification of I in the systemic circulation occurs much more readily in humans than in rats. Alternatively, II may be eliminated much more slowly (relative to the parent compound) in humans than in rats.

In summary, we believe the oral bis(2-ethylhexyl) phthalate study in the rat was a reasonable first step in elucidating the potential *in vivo* effects of I on hepatic drug metabolism during human exposure. The results generated from that study are still indicative, albeit in a qualitative fashion, of the probable influence of I on antipyrine disposition in renal failure patients. However, given the differences in the pharmacokinetic and metabolic characteristics of I between the rat and human, we now conclude that further investigations in other animal species are needed to establish a valid experimental model for assessing the biological effects of I.

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