

## Rebuttal on Influence of the Chemical Environment on Metolachlor Conformations

*Sir:* We wish to thank Buser et al. for their interest in our work presented in Jayasundera et al. (1999). In our paper we used  $^1\text{H}$  NMR and molecular mechanics to describe the different solution conformations of metolachlor isomers, and we used  $^1\text{H}$  NMR to investigate whether multiple solvents/solvent mixtures could lower the barrier for free rotation at the phenyl–N bond of metolachlor at room temperature. Our results showed that metolachlor isomers can exist in multiple conformations and that in very specific chemical environments metolachlor diastereomers *aS*, *12S* (*aR*, *12R*) and *aR*, *12S* (*aS*, *12R*) can interchange.

In the first half of our paper we discussed the metolachlor conformations and their assignments to the  $^1\text{H}$  NMR spectra in different solvents. The different metolachlor conformations (gas phase) were determined using MM+ force fields in molecular mechanics for both *S*- and *R*-chiral molecules by varying the dihedral angles and geometry optimization to find a local minimum on the potential energy surface. For each of the four metolachlor isomers (*aS*, *12S*; *aR*, *12R*; *aR*, *12S*; and *aS*, *12R*), three different conformations resulting from three stable conformations of the isopropyl methyl ether substituent were obtained. The preferred solution conformations of the metolachlor isomers were determined using  $^1\text{H}$  NMR experiments. The *aS*, *12S*/*aR*, *12R* and *aR*, *12S*/*aS*, *12R* isomers were assigned to the high (upfield) and low (downfield) intensity signals of Ar–CH<sub>3</sub>, respectively, in the  $^1\text{H}$  NMR spectra as in Moser et al. (1982) and through personal communication with the authors. The *aS*, *12S*/*aR*, *12R* to *aR*, *12S*/*aS*, *12R* ratio was 2:1 in the mixture. The preferred solution conformations of metolachlor atropisomers *aS*, *12S* (*aR*, *12R*) and *aR*, *12S* (*aS*, *12R*) were determined using 1D NOE and 2D NOESY experiments to detect the closeness in space between the isopropyl methyl ether substituent and the aromatic substituents in Bz-*d*<sub>6</sub>. Buser et al. claim that our assignments of the conformations of *aS* and *aR* metolachlor are unclear since “the *aS*/*aR* assignments are incorrectly given...”. It is not clear as to which assignments Buser et al. are referring: (1) the structural assignment of *aS* and *aR* in Figure 1 of Jayasundera et al. (1999) or (2) the  $^1\text{H}$  NMR assignment of the *aS* and *aR* atropisomers.

The chiral configurations are consistent with IUPAC sequence rule of *R,S* assignments and published research (Moser et al., 1982; Buser and Muller, 1995). The  $^1\text{H}$  NMR assignments are based on the original work by Moser et al. (1982). If these previous assignments are incorrect, they must be published separately in a peer-reviewed paper.

On the basis of the 2:1 ratio of *aS*, *12S*/*aR*, *12R* and *aR*, *12S*/*aS*, *12R* isomers and NOE data we explained the different  $^1\text{H}$  NMR frequencies corresponding to the different structural moieties of metolachlor. For the –CH<sub>2</sub>Cl protons, four frequencies of unequal intensities were observed in DMSO-*d*<sub>6</sub> and Bz-*d*<sub>6</sub>, whereas only two

frequencies were observed in CDCl<sub>3</sub>. Buser et al. claim that the four frequencies of unequal intensities of the –CH<sub>2</sub>Cl protons obtained in DMSO-*d*<sub>6</sub> and Bz-*d*<sub>6</sub> are due to the inner lines of two methylene proton AB patterns. We do not agree with Buser et al., and the spectra in Figure 2 (Jayasundera et al., 1999) clearly do not indicate an AB pattern because AB patterns are equally symmetrical to the left and right of the central peaks. On the basis of peak intensities and integrations, we hypothesize different conformers of the methylene protons.

In the second half of our paper we presented our findings on the behavior of metolachlor isomers in binary solvent mixtures. Due to the hindered rotation about the Ar–N bond, metolachlor atropisomers are generally considered to be stable at ambient temperatures (Moser et al., 1982). In our study we showed that metolachlor atropisomers were able to interchange at 25 °C in a solvent mixture of DMSO-*d*<sub>6</sub>/Bz-*d*<sub>6</sub> (60:40) by observing a coalescence of the  $^1\text{H}$  NMR peaks of *aS*, *12S*/*aR*, *12R* and *aR*, *12S*/*aS*, *12R*. Buser et al. have disputed our finding due to the hindered rotation about the Ar–N bond (Buser and Müller, 1995; Müller and Buser, 1995; Aga et al., 1999; Buser et al., 2000). We concur that the metolachlor atropisomers are conformationally stable at ambient temperatures under most environmental conditions. We respectfully disagree, however, with the prediction of Buser et al. of the “preferred” mole ratio of isomers. Reversible chemical exchanges between metolachlor diastereomers do not inherently form an equimolar mixture. For enantiomers, excess *R*-isomers over time form the *S*-isomer. In this case, however, the structures are not enantiomers but diastereomers.

The expectation that a 1:1 diastereomeric composition will remain a 1:1 composition over time and that the mole ratio of the isomers will be 1:1:1:1 in the solution state (Buser and Muller, 1995) is based only upon  $\Delta S$  (change in entropy) assumptions (Schmidt et al., 1988). The change in enthalpy ( $\Delta H$ ) is also a component in the prediction of the change in free energy of reactions ( $\Delta G$ ). For example, in the case of the structurally relatively simple diastereomer ephedrine, the  $\Delta H_f$  for the *R,S* isomer is 3.1 kcal mol<sup>-1</sup> (13 kJ mol<sup>-1</sup>), but for the *R,R* isomer, it is 7.3 kcal mol<sup>-1</sup> (30 kJ mol<sup>-1</sup>) (Schmidt et al., 1988). The *R,R* and *S,S* isomers become *R,S* and *S,R* over time. Although *R,S* and *S,R* isomers can form *R,R* and *S,S* isomers, this is a much slower process. The  $\Delta S$  argument implies if one waits long enough, independent of  $\Delta G$ , a 100 mg of a mixture of *R,S* and *S,R* isomers will form 25 mg of each of the four isomers.

Although one can begin with a 1:1:1:1 ratio of the four metolachlor isomers at 200 °C, thermodynamically it does not prove that, for example, five years from now the same ratio will be present in the vapor at a lower temperature or in a solution phase. Precisely the same transition/activation state that is postulated to slow the conversion to a 1:1:1:1 ratio from a different mole ratio also would slow the conversion of a 1:1:1:1 ratio to another mole ratio, which would have a lower free

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energy. Unequal  $\Delta H_f$  values among the four isomers from differential scanning calorimetry experiments can provide evidence that the four isomers are not equally likely to occur (Schmidt et al., 1988). Thermodynamics determines which set of diastereomers will increase over time (e.g., *SS/RR* or *RS/SR*).

The fact that metolachlor can show "varied ( $\approx 1:1$  to  $2:1$ ) diastereomer composition" in different batches (Buser and Muller, 1995; Aga et al., 1999) is unambiguous evidence that the chemical environment can change isomeric ratios. The different conditions during synthesis can include the different solvents used in the synthesis. The incorrect assumption inherent in Buser et al. is that the 1:1 ratio would occur at room temperature if the barrier to free rotation were removed. Clearly different preferred ratios could and do exist between metolachlor diastereomers in the vapor phase and in the solution phase. The energy barrier to free rotation affects only the rate of interconversion and not the final preferred ratio. Chemical environments can favor specific conformations of chiral compounds, and other researchers (Eguchi et al., 1999) have also observed solvent-influenced atropisomerism. Specific chemical environments, which lower the barrier to free rotation, do not alter the preferred ratio within that chemical environment. Under conditions that do alter the population ratios, however, these changes will occur more frequently if the energy barrier to free rotation is lower.

The energy barrier to rotation around the Ar–N bond of metolachlor was determined by Moser et al. (1982) in DMSO. It does not measure the rotational barrier in the absence of solvent nor for that matter in any other solvent. Other solvents are no longer liquid at 200 °C. Buser et al. observed rapid interconversion between the metolachlor atropisomers at 200 °C at a rate constant of  $k = 0.04 \text{ min}^{-1}$  ( $6.7 \times 10^{-4} \text{ s}^{-1}$ ), which we assume was conducted with metolachlor in DMSO. In the solvent-influenced atropisomerism observed in our experiments the rate constant was determined as  $k = 61.8 \text{ s}^{-1}$ . This is a factor of  $10^5$  difference and not a  $10^{14}$  difference as claimed by Buser et al. Rates calculated from coalescence of NMR peaks are calculated identically whether isomerization occurred due to solvent effects or due to elevated temperatures. The rate of isomerization calculated for atrazine at 150 °C was  $95 \text{ s}^{-1}$  (Welhouse and Bleam, 1992) and is also a factor of  $10^5$  different.

Broad resonance lines were observed in our data due to the coalescence of the individual peaks of the metolachlor isomers, *aS, 12S (aR, 12R)* and *aR, 12S (aS, 12R)*, as a result of rapid interconversion between them in a DMSO/benzene (60:40) solvent mixture. All of the metolachlor–solvent mixture samples, with and without  $\text{D}_2\text{O}$  or  $\text{H}_2\text{O}$ , were adequately mixed in the same way prior to NMR measurements. Buser et al. claim that the observed coalescence is due to incomplete solvent mixing because vigorous shaking of the mixture prior to NMR measurements did not reproduce the spectra in Figure 4. However, they were able to obtain the same spectra as in Figure 4 when the samples were not vigorously shaken prior to analysis.

Buser et al. further disputed the coalescence phenomena of metolachlor in the DMSO/benzene mixture due to the lack of a sharp quintet signal in the NMR spectra at 2.5 ppm for DMSO because DMSO is not expected to be involved in the coalescence. However, the H-9 proton signals of metolachlor also occur around 2.5 ppm and coalesce in the DMSO/benzene solvent mixture. Therefore, the broad signal observed at 2.5 ppm is the result of the DMSO signal as well as the coalesced H-9 protons.

Our study showed that reversible chemical exchanges between metolachlor diastereomers could occur under very specific and precise chemical (solvent) environments. However, at this point our data suggest that the rate of chemical exchange of metolachlor in natural environmental conditions would be slow due to the presence of water. Further research is needed to determine the preferred conformation/configuration of metolachlor in natural environments and the conditions and rates under which these changes occur.

#### LITERATURE CITED

- Aga, D. S.; Heberle, S.; Rentsch, D.; Hany, R.; Muller, St. R. Sulfonic and oxanilic acid meabolites of acetanilide herbicides: Separation of diastereomers and enantiomers by capillary zone electrophoresis and identification by  $^1\text{H}$  NMR spectroscopy. *Environ. Sci. Technol.* **1999**, *33*, 3462–3468.
- Buser, H.; Muller, M. D. Environmental behavior of acetamide pesticide stereoisomers. 1. Stereo- and enantioselective determination using chiral high-resolution gas chromatography and chiral high performance liquid chromatography. *Environ. Sci. Technol.* **1995**, *29*, 2023–2030.
- Buser, H. R.; Poiger, T.; Muller, M. D. Changed enantiomer composition of metolachlor in surface water following the introduction of the enantiomerically enriched product to the market. *Environ. Sci. Technol.* **2000**, in press.
- Eguchi, T.; Kondo, K.; Kakinuma, K.; Uekusa, H.; Ohashi, Y.; Mizoue, K.; Qiao, Y. Unique solvent-dependent atropisomerism of a novel cytotoxic naphthoxanthene antibiotic FD-594. *J. Org. Chem.* **1999**, *64*, 5371–5376.
- Jayasundera, S.; Schmidt, W. F.; Hapeman, C. J.; Torrents, A. Influence of the chemical environment on metolachlor conformations. *J. Agric. Food Chem.* **1999**, *47*, 4435–4442.
- Moser, H.; Rihs, G.; Sauter, H. The influence of atropisomerism and chiral center on the biological activity of metolachlor. *Z. Naturforsch.* **1982**, *87B*, 451–462.
- Müller, M. D.; Buser, H. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environ. Sci. Technol.* **1995**, *29*, 2031–2037.
- Schmidt, W. F.; Porter, W.; Carstensen, J. T. Thermodynamics in the prediction of the solubilities of binary mixtures of the diastereoisomers and enantiomers of ephedrine. *Pharm. Res.* **1988**, *5* (6), 391–395.
- Welhouse, G. J.; Bleam, W. F. NMR spectroscopic investigation of hydrogen bonding in atrazine. *Environ. Sci. Technol.* **1992**, *26*, 959–964.

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