European Journal of Soil Science, August 2007, **58,** 882–888 doi: 10.1111/j.1365-2389.2007.00927.x

The ^{15}N-CPMAS spectra of simazine and its metabolites: measurements and quantum chemical calculations

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Summary

DFT calculations are a powerful tool to support NMR studies of xenobiotics such as decomposition studies in soil. They can help interpret spectra of bound residues, for example, by predicting shifts for possible model bonds. The described bound-residue models supported the hypothesis of a free amino side chain already suspected by comparison with the experimental data of the standards. No match was found between the calculated shifts of amide bondings of the amino side chains (free or substituted) and the experimental NMR shifts of a previous study. In the present paper, first-principles quantum chemical calculations were used to support and check the interpretation of the ^{15}N cross polarization-magic angle spinning nuclear magnetic resonance (^{15}N-CPMAS NMR) spectra of simazine and its metabolites. Density functional theory (DFT) calculations were performed using Gaussian 03 and the nuclear magnetic shielding tensors were calculated using the Gauge-Independent Atomic Orbital (GIAO) method and B3LYP/6–311+G(2d,p) model chemistry. Good agreement was reached between the calculated and measured chemical shifts of the core nitrogens and the lactam and lactim forms of the hydroxylated metabolites could be clearly distinguished. The calculated spectra showed that these metabolites exist preferentially in the lactam form, an important fact when considering the possible interactions of such hydroxylated metabolites with the soil matrix. Although the calculated bound-residue models in the present study only partly matched the experimental data, they were nevertheless useful in helping to interpret the experimental NMR results of a previous study. To get a better match between the calculated and the measured shifts of the side-chain nitrogens the calculations need to be further developed, taking into account the influence of neighbouring molecules in the solid state. Altogether, quantum chemical calculations are very helpful in the interpretation of NMR spectra. In the future, they can also be very useful for the prediction of NMR shifts, in particular when it is not possible to measure the metabolites due to a lack of material or in cases where practical experiments cannot be conducted.

Introduction

Simazine (6-chloro-N,N′-diethyl-1,3,5-triazine-2,4-diamine) (Figure 1) was introduced in the 1950s by J. R. Geigy SA (today: Syngenta) as the first triazine herbicide. It is used as a selective pre-emergence herbicide for annual dicotyledonous pest plants and weeds in deep-rooting crops such as maize or vines. Although the European regulatory authority, the Standing Committee on the Food Chain and Animal Health (SCFCAH) of the European Commission, has voted against a re-registration of simazine in Europe, along with the better known triazine representative atrazine, it is still a widely used herbicide around the world, especially in maize cultivation.

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Received 5 March 2007; revised version accepted 24 April 2007

Binding and release of a pesticide and its metabolites in soil is mainly determined by the type of interaction with soil components like organic matter or the mineral phase. Nuclear magnetic resonance (NMR) can be used for the characterization of bound residues as binding of the pesticide or its metabolites to the soil matrix causes shifts in the initial positions of the NMR signals of the pesticide. Knicker *et al*. (2001) successfully analysed ^{15}N-labelled TNT residues in soil and soil suspensions. In a previous study (Berns *et al*., 2005), ^{15}N-CPMAS-NMR was used to determine the structure of bound simazine residues in soil. First-principles quantum calculations have already been

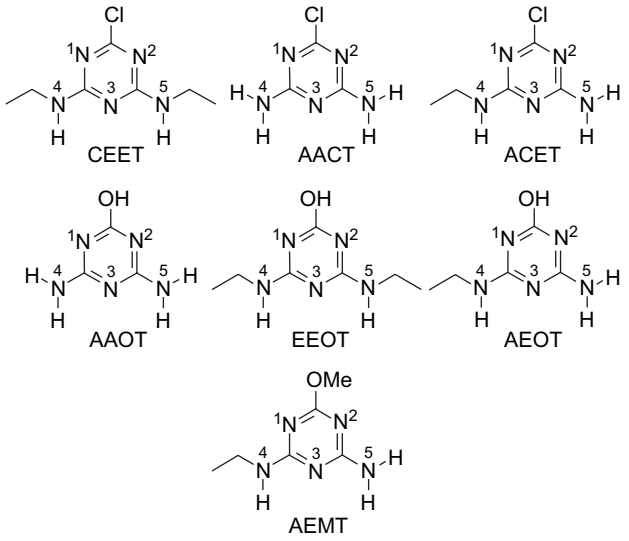

Figure 1 Simazine and its metabolites. CEET = simazine; AACT = diaminochlorotriazine; ACET = deethylsimazine; AAOT = diaminohydroxytriazine; EEOT = hydroxysimazine; AEOT = deethylhydroxysimazine; AEMT = deethylmethoxysimazine.

successfully applied to interpret, for example, the ^{13}C-NMR spectra of methabenzthiazuron and its metabolites (Koglin *et al*., 2004). Meng & Carper (2002) used GIAO NMR calculations to compare theoretical and experimental ^{1}H and ^{13}C NMR shifts of atrazine and atrazine dimers, and the Hartree-Fock (HF) and DFT calculations supported the authors' concept of hydrophobic interactions between atrazine molecules. Gobetto *et al*. (2005) were able to confirm hydrogen bonds of supramolecular adducts seen in solid-state ^{1}H and ^{15}N NMR spectra. Tuppurainen & Ruuskanen (2003) predicted ^{13}C NMR chemical shifts of chlorinated boranes with GIAO calculations and concluded that the calculated shifts needed to be empirically scaled to achieve a good numerical agreement, which was probably due to the large deshielding effect of the chlorine.

The objectives of this investigation were to clarify the interpretation of the ^{15}N-CPMAS spectra of simazine and its metabolites with the help of first-principles quantum calculations, as in some spectra, especially those of the hydroxylated metabolites, the assignments of the signals were not clear. Furthermore, an attempt was made to enhance the interpretation of the bound-residue spectra published in an earlier study (Berns *et al*., 2005) by calculating the chemical shifts of bound-residue models. NMR chemical shifts were computed using the GIAO method as implemented in Gaussian 03, which is now well established.

Materials and methods

Chemicals

^{15}N-core-labelled (99 atom-% ^{15}N) and ^{15}N-side-chain-labelled simazine (98 atom-% ^{15}N) were provided by Syngenta (Basel, Switzerland). ^{15}N-labelled glycine (minimum 99 atom-%) was obtained from Isotec (Miamisburg, USA). The non-labelled metabolites were purchased from Riedel-de Haën (Seelze, Germany) (Figure 1).

NMR experiments

Solid-state ^{15}N{^{1}H}CP-NMR spectra were obtained on an Avance DSX-500 spectrometer (Bruker, Rheinstetten, Germany) operating at 50.71 MHz. Samples were spun in a 7-mm zirconium dioxide rotor (Bruker) at 4–5 kHz. Between 200 (labelled substances) and 48360 scans (unlabelled metabolites) were acquired at repetition times of 1 s, 5 s and 7 s depending on the compound. The contact pulse and 90° pulse length were 3 ms and 7 μs, respectively. ^{15}N-labelled glycine was used as a secondary reference for the solid-state experiments and chemical shifts are reported relative to nitromethane.

Computational methods and assignments

Calculations were performed using Gaussian 03 (Frisch *et al*., 2003) implemented on the IBM p690 cluster of Forschungszentrum Jülich GmbH. DFT calculations were performed and the GIAO nuclear magnetic shielding tensors were calculated using the B3LYP functional in conjunction with the 6–311+G(2d,p) basis set. All geometries were fully optimized using the B3LYP/6–31G(d,p) method in Gaussian 03 with '*verytight*' convergence criteria until the root-mean-square forces were smaller than 1×10^{-6} hartree bohr^{-1}.

As Alkorta & Elguero (1998, 2003) and Gauss *et al*. (Gauss, 1993; Gauss & Stanton, 1995, 1996, 2000; Gauss & Werner, 2000) have already pointed out, referencing the calculated spectra to the firmly established reference substance nitromethane proved to be difficult as the geometry optimization of nitromethane is tricky. Alkorta & Elguero (2003) reported about nine different calculated values for nitromethane from different publications, ranging from –138.9 to –269.4 p.p.m., depending on the calculation method and basis set used. In this paper, the reference value of –158.03 p.p.m. for nitromethane was used as calculated by Begtrup *et al*. (1998) with the same method and basis set as used in this work. Correlation of the calculated and experimental values was done by linear regression according to Tuppurainen & Ruuskanen (2003).

Results and discussion

In general, the calculated (referenced) shifts, especially those of the side-chain nitrogens were too deshielded, but overall the correlation between the calculated and experimental values was nevertheless good (Figure 2), with a squared correlation coefficient, r^2, of 0.991 and a standard deviation of 4.1 p.p.m. Thus, interpretation of the experimental spectra of the standards was not seriously hindered.

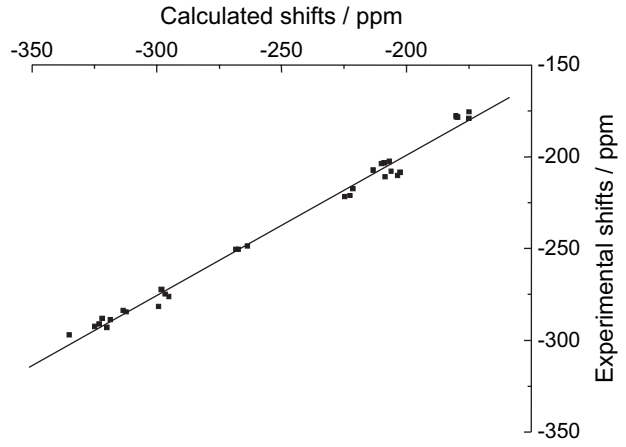

Figure 2 Correlation of calculated and experimental chemical shifts of all investigated samples (correlation equation: $\sigma_{exp} = 0.764\ \sigma_{calc} - 46.312$).

The geometry optimization with a higher basis set (6–311+G(2d,p)) did not give a better agreement between calculated and measured shifts as tested with the simazine and diaminohydroxytriazine structures. To test whether a different calculation method would give better results, especially as the pyramidal structure of the amino groups may be sensitive to a change of method, the shifts of diaminohydroxytriazine were also calculated with PW91 (Perdew & Wang's gradient-corrected correlation functional; Perdew, 1991; Perdew *et al.*, 1996). However, this did not yield a better match with the experimental values and further calculations were abandoned.

Standards

Figure 3 shows the ^{15}N-CPMAS spectra of the unlabelled, ^{15}N-core- and ^{15}N-side-chain-labelled simazine (CEET), respectively. The spectra of the two labelled versions make the interpretation of the simazine spectrum fairly easy. As expected, the large signal at –272.3 p.p.m. can be assigned to the side-chain nitrogens N4 and N5, which have a hydrogen atom attached and therefore give a strong signal due to a more effective cross polarization. The signal at –207.9 p.p.m. is assigned to the core nitrogen N3 and the signals at –177.6 and –178.2 p.p.m. are due to the core nitrogens N1 and N2. The calculated shifts are –180.1 p.p.m. for N1 and N2, –206.1 p.p.m. for N3 and –298.1 p.p.m. for the side chains. The shifts of the side-chain nitrogens are off by 25.8 p.p.m., but the relative positions of the signals to each other are confirmed by the calculations. One reason for this greater deviation of the side chain signals might be that the calculations were done on single (gaseous) molecules while in the solid the neighbouring molecules influence the chemical shift. Because of the greater proximity of the side chains to these neighbouring molecules the effect should be bigger than on the core nitrogens. Furthermore, due to the greater number of rotational and translational degrees of freedom of the side-chain nitrogens their geometry optimization (in the gas phase) is afflicted with greater difficulties than that of the core nitrogens. The NMR spectra were measured in the solid state where the number of

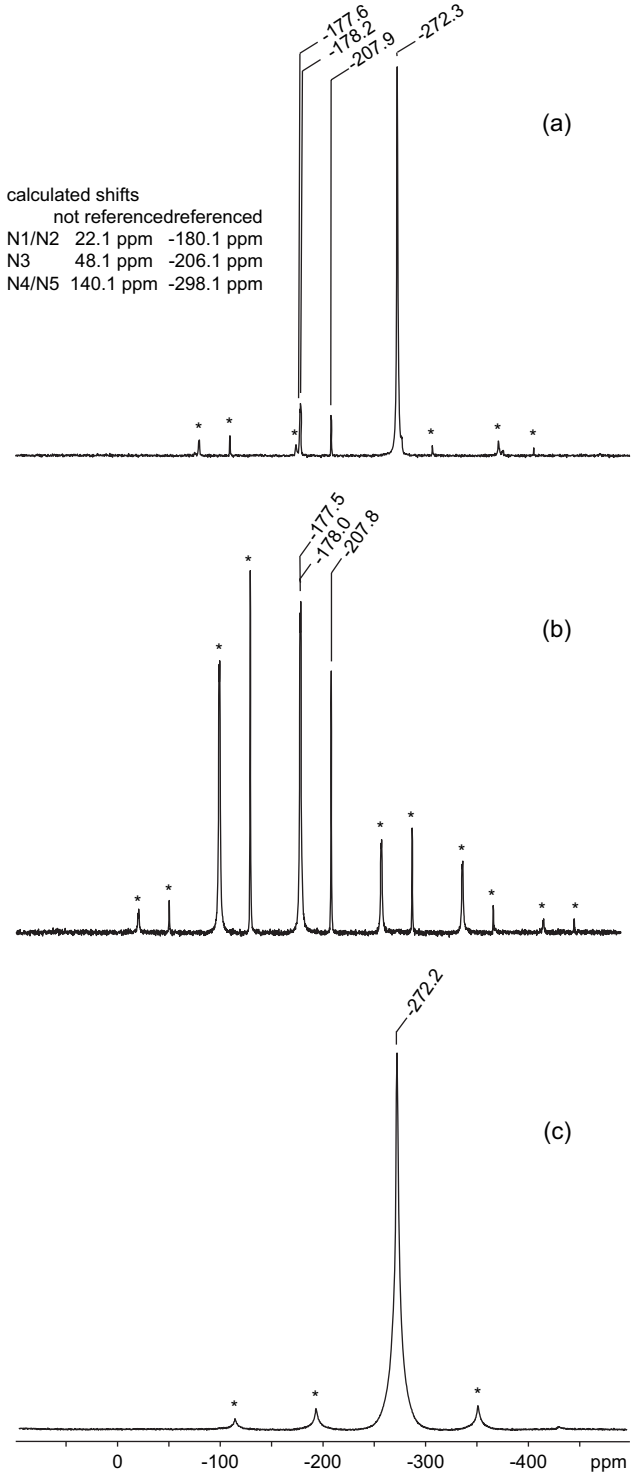

Figure 3 ^{15}N-CPMAS spectrum and calculated chemical shifts of (a) unlabelled simazine (CEET), (b) ^{15}N-core-labelled simazine, (c) ^{15}N-side-chain-labelled simazine (* = spinning side bands).

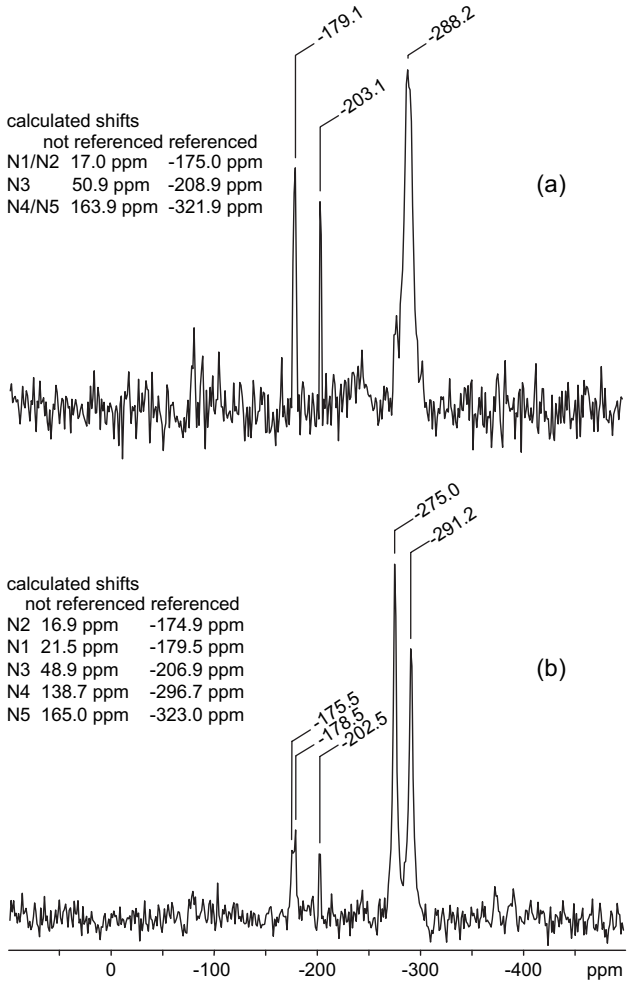

Figure 4 ^{15}N-CPMAS spectrum and calculated chemical shifts of (a) diaminochlorotriazine (AACT) and (b) deethylsimazine (ACET).

rotational and translational degrees of freedom is lower, hence leading to a larger error.

Figure 4(a) shows the ^{15}N-CPMAS spectrum of the unlabelled diaminochlorotriazine (AACT). Compared to the spectra of simazine the side-chain signal is shifted to –288.2 p.p.m. and the core nitrogen N3 signal to –203.1 p.p.m. As expected, the N1/N2 signal is only slightly shifted (–179.1 p.p.m.) as these nitrogen atoms are least influenced by changes in the side chains. DFT calculations produced chemical shifts of –175.0 p.p.m. for the N1/N2 signal, –208.9 p.p.m. for the N3 and –321.9 p.p.m. for the dealkylated side chains. The shifts of the core nitrogens match well, while the calculated shifts of the side chains are off by 33.7 p.p.m., but again the succession of the signals is confirmed by the calculated spectrum.

The ^{15}N-CPMAS spectrum of the unlabelled deethylsimazine (ACET) (Figure 4b) is comparable to the simazine spectrum, with chemical shifts of –175.5, –178.5 and –202.5 p.p.m. for the core nitrogens, except that the side-chain nitrogen signals have split up and the free amino side chain gives a signal at –291.2 p.p.m., while the alkylated side chain remains at a chemical shift of –275.0 p.p.m. The calculated shifts were –174.9, –179.5 and –206.9 p.p.m. for the core nitrogens and –296.7 and –323.0 p.p.m. for the side chains. Again the core nitrogen shifts are well reproduced by the calculations while the side-chain signals are off by 21.7 and 31.8 p.p.m., respectively.

Figure 5(a) shows the ^{15}N-CPMAS spectrum of the unlabelled diaminohydroxysimazine (AAOT). In the region of the core nitrogens two signals are measured at –208.5 and –217.5 p.p.m. and a third strong signal is measured at –248.6 p.p.m. Furthermore, the dealkylated side-chain nitrogens produce separate signals and the measured spectrum therefore suggests an asymmetric constitution of the molecule. The most common asymmetric structure for a hydroxylated triazine system is a lactam-lactim tautomerism of the hydroxy group with an adjacent nitrogen (Figure 6). Lactam-lactim tautomerism for hydroxylated triazine metabolites has already been reported by Lerch *et al*. (1997) and Skipper *et al*. (1976). Skipper *et al*. (1976) reported that precipitates of hydrolyzed atrazine

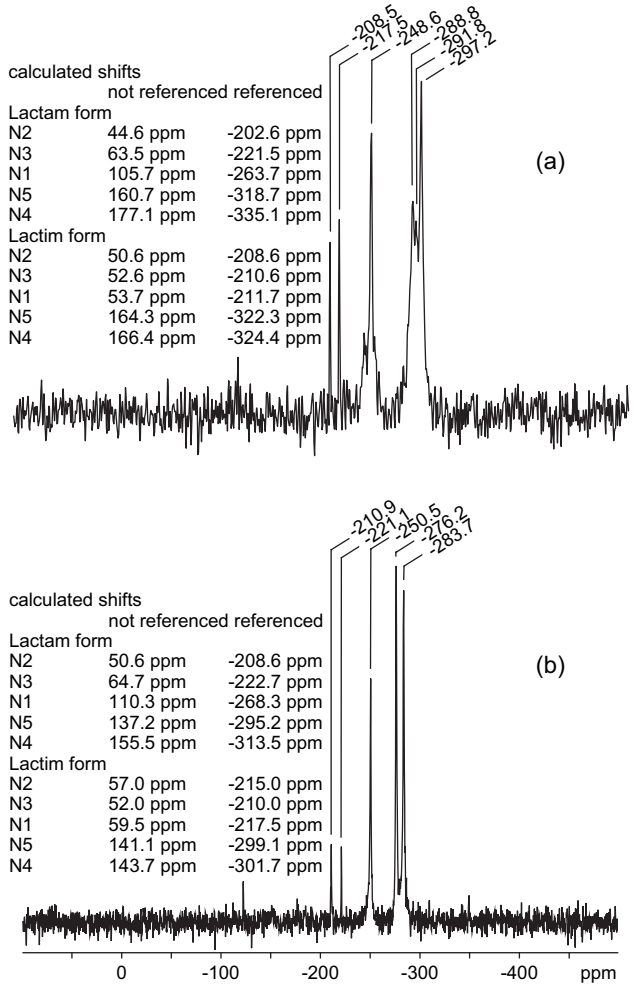

Figure 5 ^{15}N-CPMAS spectrum and calculated chemical shifts of (a) diaminohydroxytriazine (AAOT) and (b) hydroxysimazine (EEOT).

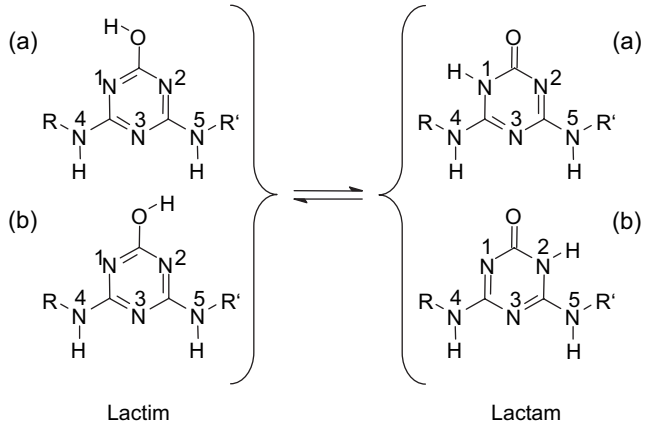

Figure 6 Lactam-lactim tautomerism with possible conformers.

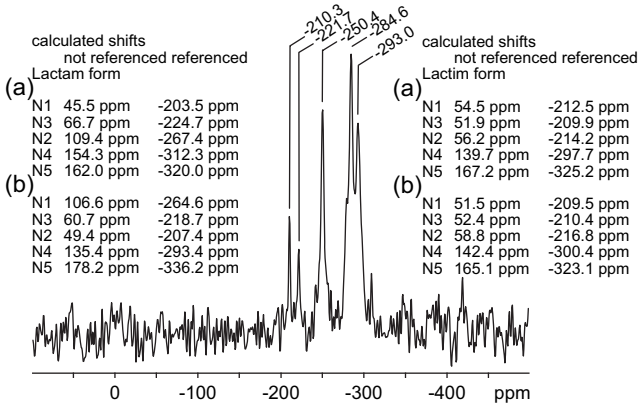

Figure 7 ^{15}N-CPMAS spectrum and calculated chemical shifts of deethylhydroxysimazine (AEOT) (a and b designate the two possible positions of the 'hydroxy-hydrogen' as shown in Figure 6 with R = ethyl and R′ = H).

prepared between pH 3.3 and 10 were similar to technical hydroxyatrazine and according to IR spectra these samples existed in the lactim form. In contrast, in the present study NMR results support the lactam form in the solid state with a hydrogen atom on the core nitrogen N1. The calculated spectrum assigned the first two signals to the core nitrogens N2 (opposite the NH-group) and N3. The signal at –248.6 p.p.m. was assigned to the core nitrogen N1 carrying the hydrogen atom. This also explains the more intense signal of the core nitrogen due to a more effective cross-polarization. The signals of the side-chain nitrogens were assigned to N5 (farthest away from the NH-group) and N4 (adjacent to the NH-group). Figure 5(b) shows the ^{15}N-CPMAS spectrum of the unlabelled hydroxysimazine (EEOT), which has a similar appearance to that of the AAOT spectrum and again the lactam form of the molecule is confirmed by the calculations. The same assignment was made as for the AAOT spectrum and the central signal at –250.5 p.p.m. was due to the core NH-group.

Figure 7 shows the ^{15}N-CPMAS spectrum of the unlabelled deethylhydroxysimazine (AEOT). Again there is a signal at –250.4 p.p.m. due to the core nitrogen N1 with an attached hydrogen. In the case of the asymmetric AEOT, two lactam and lactim forms are possible depending either on the side to which the hydroxy group is turned or which core nitrogen is carrying the hydrogen (Figure 6). The calculations showed that the conformer which came closest to the measured spectrum was the lactam version (a) where the hydrogen was attached to the core nitrogen adjacent to the ethyl-substituted side chain. The signals at –210.3 and –221.7 p.p.m. were assigned to the core nitrogens N2 and N3, respectively, and the signals at –284.6 and –293.0 p.p.m. to the side-chain nitrogens N4 and N5, respectively.

In the ^{15}N-CPMAS spectrum of the unlabelled deethylmethoxysimazine (AEMT) (Figure 8) there is no signal in the –250 p.p.m. region due to the lack of lactam-lactim tautomerism. The side-chain signals split up and the amino group shifts to –292.4 p.p.m. The spectra of AACT and ACET suggest that the signal at –203.7 p.p.m. is due to the core nitrogen N3 and the signal at –207.3 p.p.m. could be assigned to the core nitrogens N1 and N2. However, the calculation of the spectrum gives a slightly different picture. Rotation of the methoxy group gives several conformers. The calculated spectrum which came closest to the measured one was the conformer where the methoxy group was turned away from the ethyl side chain. Calculation of this conformer showed that the core nitrogens N3 and N1 were separated by only 0.3 p.p.m. The next signal was shifted further by 3.2 p.p.m. and was assigned to the core nitrogen N2 located between the methoxy and amino groups. The assumed positions of the side-chain nitrogens N4 and N5 were confirmed by the calculations.

Bound residues

To help the interpretation of the bound-residue spectra of the bound-residue study (Berns *et al*., 2005), different bound-residue

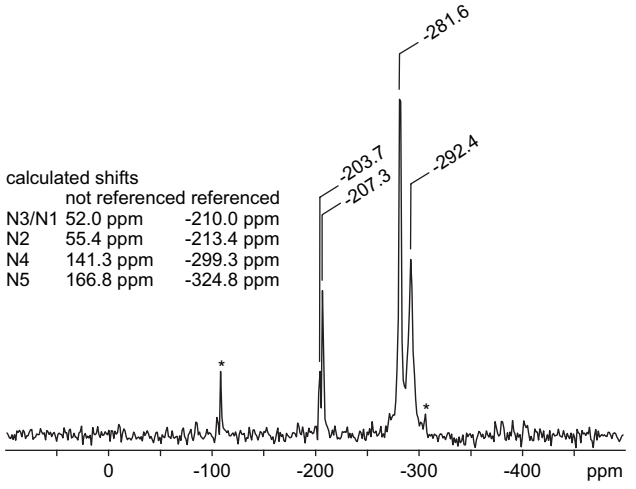

Figure 8 ^{15}N-CPMAS spectrum and calculated chemical shifts of deethylmethoxysimazine (AEMT) (* = spinning side bands).

models were calculated. Benzoic acid was used as a model for a humic acid module and four different bonding possibilities with simazine, hydroxysimazine and diaminochlorotriazine were calculated (Figure 9). As possible reactions we considered an ester bonding with the hydroxy group of hydroxysimazine and amide bondings with the amino groups of either simazine or diaminochlorotriazine. The spectra of the ^{15}N-core labelled residues showed three signals at –200.7, –182.7 and –108.3 p.p.m. and the ^{15}N-side-chain labelled residues gave one signal at –291.8 p.p.m. after 565 days of incubation (Berns *et al*., 2005). Comparison with the spectra of the standards showed that the side-chain signal at –291.8 p.p.m. was probably due to a free amino group. The calculated chemical shifts of the bound-residue models confirmed this hypothesis as the only calculated chemical shift matching the experimental data was the one of the free amino group at –321.6 p.p.m. (Figure 9c). The calculated chemical shifts of the free amino groups in the standards AACT, AAOT and ACET ranged from –312.9 to –324.4 p.p.m., which corresponds to experimental shifts from –288 to –297 p.p.m., respectively (using the regression equation of Figure 2). In the bound-residue models no other bonding possibility resulted in a theoretical chemical shift in this region. Amino ethyl side chains only slightly changed their chemical shift in these residue models if not involved directly in a new bonding. A bonding of the amino group to benzoic acid resulted in chemical shifts of –237.4 p.p.m. in the case of the ethyl substituted amino group (Figure 9a) and –253 p.p.m. in the case of the non-substituted amino group (Figure 9c, d). These values correspond to experimental shifts of about –228 and –240 p.p.m., respectively, and neither of them were visible in the experimental spectrum of the side-chain labelled bound residues. Of course it must be taken into account that the signals in the experimental spectra are weak and the absence of a signal does not imply the absence of the corresponding bonding. Nevertheless the presence of a free amino group can be affirmed. In the spectrum of the core-labelled bound residues the signal at –108.3 p.p.m. lies in the region of nitrile groups and is an indication of a degradation of the core followed by an integration into the organic matter in the form of nitrile groups. This is confirmed by the observation that none of the described models showed a calculated chemical shift in this region. The greatest change in chemical shift of the core nitrogens was found in the bound-residue model of two benzoic acids with diaminochlorotriazine, where two of the core nitrogens had a calculated chemical shift of –146.7 p.p.m. (Figure 9d), corresponding to an experimental chemical shift of about –158 p.p.m. Although there are a lot more possibilities for bound residues, it seems clear that an intact triazine ring does not produce a signal in the region of –108.3 p.p.m., regardless of the substituents. Therefore the degradation of the core is the most likely explanation for the existence of this signal. The signals at –182.7 and –200.7 p.p.m. in the spectrum of the core-labelled bound residues also indicate the presence of intact triazine rings. Comparison with the spectra of the standards shows that these signals are most likely not due to free hydroxylated metabolites, as the typical signal at around –250 p.p.m. of the core nitrogen N3 in the lactam form is missing. Calculation of a bound-residue model with an ester bond between hydroxysimazine in its lactim constitution and benzoic acid (Figure 9b) gave calculated chemical shifts of –192.5, –197.7 and –203.4 p.p.m., corresponding to experimental shifts of about –193, –197 and –202 p.p.m., respectively. From the calculated bound-residue models these are the values that are closest to the experimental values. The models with amide bonding on the side chains give calculated chemical shifts that are far off the experimental values. From the calculated bound-residue models it must be concluded that amide bonding of the side chains to carboxylic groups of the organic matter is not a major factor in the formation of simazine residues. The closeness of the hydroxylated bound-residue model to the experimental values suggests hydroxylated bound residues, though not in this form. A possible model, not calculated in this paper, might be a mixed-mode binding of hydrophobic interaction and cation exchange of hydroxylated metabolites, as proposed by Lerch *et al*. (1997).

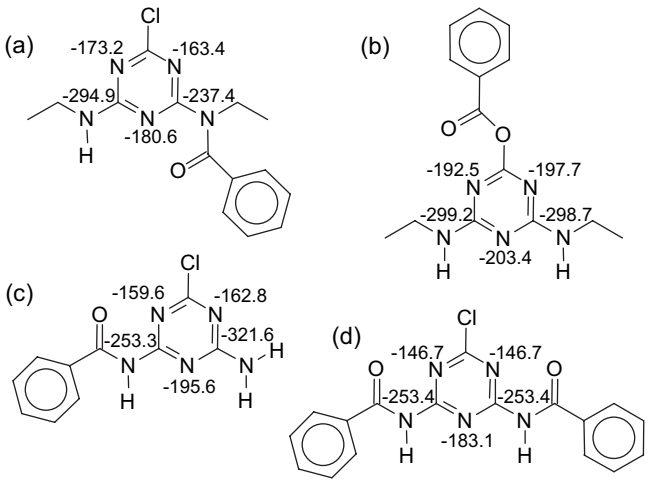

Figure 9 Calculated referenced chemical shifts of bound-residue models with benzoic acid as humic acid module. (a) amide bonding of simazine with benzoic acid, (b) ester bonding of hydroxysimazine with benzoic acid, (c) amide bonding of diaminochlorotriazine with one benzoic acid module, (d) amide bonding of diaminochlorotriazine with two benzoic acid modules.

Conclusions

This study showed that the interpretation of NMR spectra can be meaningfully complemented with first-principles quantum calculations. The assignments in the spectra of simazine and its metabolites could be confirmed by the calculations and, especially with the core nitrogen signals, a good match could be achieved. The

calculations could clearly distinguish between the lactam and lactim form of the hydroxylated metabolites and showed that they are present in the lactam form and not in the lactim form as usually displayed, even though the lactam form destroys the heteroaromatic triazine system. The fact that hydroxylated metabolites exist preferentially in the lactam form has to be taken into account when considering possible interactions of these metabolites with the soil matrix, as Lerch *et al*. (1997) did in their bound-residue study of hydroxylated atrazine.

DFT calculations are a powerful tool to support NMR studies of xenobiotics such as decomposition studies in soil. They can help interpret spectra of bound residues, for example, by predicting shifts for possible model bonds. The described bound-residue models supported the hypothesis of a free amino side chain already suspected by comparison with the experimental data of the standards. No match was found between the calculated shifts of amide bondings of the amino side chains (free or substituted) and the experimental NMR shifts of a previous study. The calculated shifts of the hydroxylated bound-residue model did not exactly match the experimental NMR shifts, but through their closeness they suggested hydroxylated bound residues though not in the presented form.

For predictions of possible shifts the calculations need to be further developed, especially as the signals of the side-chain nitrogens are still difficult to predict. This is probably due to interactions with neighbouring molecules in the solid which are not taken into account in the DFT calculations of single molecules *in vacuo*. This effect is less strong for the core nitrogen where the calculations are in good agreement with the measured data.

In the future, calculations of the spectra of metabolites can be very helpful, in particular when it is not possible to measure the metabolites due to a lack of material. Kubicki (2005) used molecular modelling to assess the bioavailability of organic contaminants and pointed out that molecular modelling can help to predict metabolic intermediates as well as extrapolate their environmental behaviour in cases where practical experiments cannot be conducted.

Acknowledgement

We thank Syngenta (Basel, Switzerland) for the labelled substances.

References

Alkorta, I. & Elguero, J. 1998. Ab initio (GIAO) calculations of absolute nuclear shieldings for representative compounds containing (1(2)H, (6(7))Li, B-11, C-13, (14(15))N, O-17, F-19, Si-29, P-31, S-33, and Cl-35 nuclei. *Structural Chemistry*, **9,** 187–202.

Alkorta, I. & Elguero, J. 2003. GIAO calculations of chemical shifts in heterocyclic compounds. *Structural Chemistry*, **14,** 377–389.

Begtrup, M., Balle, T., Claramunt, R.M., Sanz, D., Jimenez, J.A., Mo, O. *et al*. 1998. GIAO ab initio calculations of nuclear shieldings of monosubstituted benzenes and N-substituted pyrazoles. *Journal of Molecular Structure Theochem*, **453,** 255–273.

Berns, A., Vinken, R., Bertmer, M., Breitschwerdt, A. & Schäffer, A. 2005. Use of N-15-depleted artificial compost in bound residue studies. *Chemosphere*, **59,** 649–658.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R. *et al*. 2003. *Gaussian 03, Revision B.03*. Gaussian, Inc, Pittsburgh, PA.

Gauss, J. 1993. Effects of electron correlation in the calculation of nuclear-magnetic-resonance chemical-shifts. *Journal of Chemical Physics*, **99,** 3629–3643.

Gauss, J. & Stanton, J.F. 1995. Coupled-cluster calculations of nuclear-magnetic-resonance chemical-shifts. *Journal of Chemical Physics*, **103,** 3561–3577.

Gauss, J. & Stanton, J.F. 1996. Perturbative treatment of triple excitations in coupled-cluster calculations of nuclear magnetic shielding constants. *Journal of Chemical Physics*, **104,** 2574–2583.

Gauss, J. & Stanton, J.F. 2000. Analytic first and second derivatives for the CCSDT-n (n = 1–3) models: a first step towards the efficient calculation of CCSDT properties. *Physical Chemistry Chemical Physics*, **2,** 2047–2060.

Gauss, J. & Werner, H.J. 2000. NMR chemical shift calculations within local correlation methods: the GIAO-LMP2 approach. *Physical Chemistry Chemical Physics*, **2,** 2083–2090.

Gobetto, R., Nervi, C., Valfre, E., Chierotti, M.R., Braga, D., Maini, L. *et al*. 2005. H-1 MAS, N-15 CPMAS, and DFT investigation of hydrogen-bonded supramolecular adducts between the diamine 1,4-diazabicyclo-[2.2.2]octane and dicarboxylic acids of variable chain length. *Chemistry of Materials*, **17,** 1457–1466.

Knicker, H., Achtnich, C. & Lenke, H. 2001. Solid-state nitrogen-15 nuclear magnetic resonance analysis of biologically reduced 2,4,6-trinitrotoluene in a soil slurry remediation. *Journal of Environmental Quality*, **30,** 403–410.

Koglin, E., Witte, E.G., Willbold, S. & Meier, R.J. 2004. Evaluation of ab initio methods for the calculation of C-13 NMR shifts of metabolites of methabenzthiaruzon. *Journal of Molecular Structure – Theochem*, **712,** 233–237.

Kubicki, J.D. 2005. Computational chemistry applied to studies of organic contaminants in the environment: examples based on benzo[a]pyrene. *American Journal of Science*, **305,** 621–644.

Lerch, R.N., Thurman, E.M. & Kruger, E.L. 1997. Mixed-mode sorption of hydroxylated atrazine degradation products to soil: a mechanism for bound residue. *Environmental Science and Technology*, **31,** 1539–1546.

Meng, Z.H. & Carper, W.R. 2002. GIAO NMR calculations for atrazine and atrazine dimers: comparison of theoretical and experimental H-1 and C-13 chemical shifts. *Journal of Molecular Structure – Theochem*, **588,** 45–53.

Perdew, J.P. 1991. Unified theory of exchange and correlation beyond the local density approximation. In: *Electronic Structure of Solids '91* (eds P. Ziesche & H. Eschrig), pp. 11–20. Akademie Verlag, Berlin.

Perdew, J.P., Burke, K. & Wang, Y. 1996. Generalized gradient approximation for the exchange-correlation hole of a many-electron system. *Physical Review B*, **54,** 16533–16539.

Skipper, H.D., Volk, V.V. & Frech, R. 1976. Hydrolysis of a chloro-S-triazine herbicide. *Journal of Agricultural and Food Chemistry*, **24,** 126–129.

Tuppurainen, K. & Ruuskanen, J. 2003. NMR and molecular modeling in environmental chemistry: prediction of C-13 chemical shifts in selected C-10-chloroterpenes employing DFT/GIAO theory. *Chemosphere*, **50,** 603–609.