

# Deuterium-Labeled Lipids as Structural Probes in Liquid Crystalline Bilayers. A Deuterium Magnetic Resonance Study

Joachim Seelig\* and Werner Niederberger

Contribution from the Biocenter of the University of Basel,  
Department of Biophysical Chemistry, Basel, Switzerland.  
Received September 7, 1973

**Abstract:** Selectively deuterated lipids are used as building stones for liquid crystalline bilayers. The deuterium magnetic resonance signal is found to be a doublet as a consequence of the anisotropic motion of the lipid molecules. From the separation of the lines the order parameter at different positions in the bilayer is easily evaluated. Similar to spin label probes the deuterated lipids detect a flexibility gradient in the bilayer, but quantitatively the two methods differ by about 20 to 30%. The deuterium results seem more reliable since deuterium labels do not perturb the bilayer structure.

The advent of fast-Fourier-transform (FFT) techniques has made it possible to detect deuterium magnetic resonance (dmr) signals even at the natural abundance of deuterium.<sup>1</sup> The purpose of this report is to illustrate that dmr can provide structural information which is equivalent or even surpasses that obtained by the spin label epr method<sup>2</sup> and which is not readily available from measurements of other nuclei such as <sup>1</sup>H or <sup>13</sup>C. This will be shown below for the motion of deuterium-labeled lipids in oriented samples of liquid crystalline bilayers.

## Theory

Let us briefly define our problem. Two questions may be formulated in considering a cross section of a lipid bilayer. (1) How are the hydrocarbon chains oriented at various positions in the bilayer? (2) How fast are the motions of the chain segments? The first question asks for the *structure* of the bilayer, the second for its *dynamical properties*. These questions can be rephrased in terms of two experimental parameters that describe structure and dynamics as follows: (1) what are the order parameters  $S_{ii}$  and (2) what are the correlation times  $\tau_i$  at various positions in a lipid bilayer?

The spin label method has provided a particularly simple approach to the solution of the structural problem, since the order parameters can be evaluated without much computational effort from the relative positions of the epr lines.<sup>3,4</sup> However, the relevance of such results has been criticized on grounds that the nitroxide moiety introduces too big a perturbation into the bilayer.<sup>5,6</sup> Nmr measurements of <sup>1</sup>H or <sup>13</sup>C nuclei

avoid the problem of perturbing the system, but here the difficulties are shifted to the quantitative relation of  $T_1$  and  $T_2$  relaxation times to relevant molecular parameters.<sup>7-9</sup> Stimulated by the work of Oldfield, *et al.*, using perdeuterated lipids<sup>10,11</sup> and by an earlier paper by Diehl,<sup>12</sup> we felt that the synthesis of selectively deuterated lipids combined with dmr could bridge the gap between the spin label method and nmr techniques. Dmr of selectively deuterated molecules has the following advantages. (1) By means of appropriate chemical synthesis the deuterium atom can be attached to practically any position in a molecule. In this way differences between the various parts of the molecule can be observed. This is analogous to the spin label method with the additional advantage that the replacement of hydrogen by deuterium causes no structural changes in the molecule. (2) The deuterium nmr signal of the labeled molecule is the only signal in the spectrum and can be assigned unambiguously. (3) Dmr of deuterium labels easily detects anisotropic motions. For a rapid isotropic motion the dmr spectrum consists of one single line. On the other hand, if the motion is anisotropic, the signal will be a doublet due to the quadrupole moment of the deuterium nucleus. The frequency difference,  $\Delta\nu$ , between the two lines depends on the anisotropy of motion and on the orientation of the axis of motional averaging with respect to the magnetic field. For a homogeneously oriented sample of lipid bilayers  $\Delta\nu(\theta)$  is given by

$$\Delta\nu(\theta) = (3/4)(e^2qQ/h)S(3 \cos^2 \theta - 1) \quad (1)$$

where  $\theta$  denotes the angle between the bilayer normal and the magnetic field and  $S$  is the order parameter of the deuterium bond, *i.e.*, the C-D bond in our case. Since the quadrupole splitting constant ( $e^2qQ/h$ ) for the C-D bond has been determined in paraffins to be 170

(1) J. M. Briggs, L. F. Farnell, and E. N. Randall, *J. Chem. Soc., Chem. Commun.*, 70 (1973).

(2) Reviews of the spin label technique: H. M. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, 3, 91 (1970); P. Jost, A. S. Waggoner, and O. H. Griffith in "Structure and Function of Biological Membranes," L. Rothfield, Ed., Academic Press, New York, N. Y., 1971; I. C. P. Smith in "Biological Applications of Electron Spin Resonance," H. M. Swartz, J. R. Bolton, and D. C. Borg, Ed., Wiley, New York, N. Y., 1972.

(3) J. Seelig, *J. Amer. Chem. Soc.*, 92, 3881 (1970).

(4) W. L. Hubbell and H. M. McConnell, *J. Amer. Chem. Soc.*, 93, 314 (1971).

(5) A. D. Keith and R. J. Mehlhorn, *Chem. Phys. Lipids*, 8, 314 (1972).

(6) D. A. Cadenhead and F. Müller-Landau, *Biochim. Biophys. Acta*, 307, 279 (1973).

(7) S. I. Chan, C. H. A. Seiter, and G. W. Feigenson, *Biochem. Biophys. Res. Commun.*, 46, 1448 (1972).

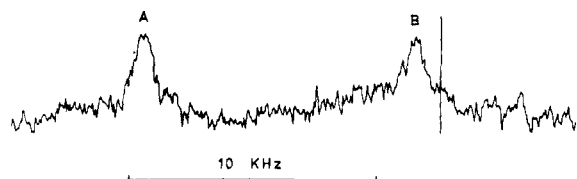
(8) A. F. Horwitz, D. Michaelson, and M. P. Klein, *Biochim. Biophys. Acta*, 298, 1 (1973).

(9) Y. K. Levine, P. Partington, and G. C. K. Roberts, *Mol. Phys.*, 25, 497 (1973).

(10) E. Oldfield, D. Chapman, and W. Derbyshire, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 16, 102 (1971).

(11) E. Oldfield, D. Chapman, and W. Derbyshire, *Chem. Phys. Lipids*, 9, 69 (1972).

(12) P. Diehl and Th. Leipert, *Helv. Chim. Acta*, 47, 545 (1964).



**Figure 1.**  $^2\text{H}$  nmr spectrum of a random distribution of liquid crystalline bilayers. Phase composition: 1,1-dideuteriooctanol (30 wt %), sodium octanoate (34 wt %), and water (36 wt %). Liquid crystalline material (500 mg) was brought into a 10-mm nmr sample tube and 24,000 free induction decays were accumulated using  $90^\circ$  pulses of  $20\ \mu\text{sec}$  (2K/1K spectrum, total accumulation time 16 min). The vertical line indicates the center of the spectrum ( $\nu = 13.81\ \text{MHz}$ ). Signals A and B should be located symmetrically around the center. Due to computer limitations (maximum spectral width 24 kHz) signal B is folded back. The true separation between A and B amounts to  $\Delta\nu_r = 24.2 \pm 0.2\ \text{kHz}$ . All spectra were obtained on a Bruker HX-90 spectrometer operating at 21.1 kG and equipped with a BNC 20 K-FFT computer. A  $^{19}\text{F}$  field frequency locking system was employed. Measurements were made at room temperature ( $\sim 28^\circ$ ).

kHz,<sup>13</sup> the order parameter  $S_{\text{CD}}$  can easily be evaluated from eq 1. If the bilayers are not oriented, but distributed at random, the observed spectrum consists of two rather broad peaks, which arise from a superposition of a large number of resonance signals with different splittings. The separation of the two maxima is then given by

$$\Delta\nu_r = \left(\frac{3}{4}\right)(e^2qQ/h)S \quad (2)$$

## Results

The essential feature of our experiments is therefore to replace one of the bilayer building stones by its selectively deuterated analog and to measure the residual deuterium quadrupole splitting. As bilayer systems we have chosen lyotropic liquid crystals composed of fatty acids (sodium salt), long chain alcohols, and water and previously studied by us using spin labels.<sup>14</sup> The phase diagrams of such ternary mixtures are known from X-ray diffraction spectroscopy.<sup>15</sup> In a certain concentration range the lipid molecules are arranged in bilayers, which are separated from each other by layers of water. Both amphiphilic components, the fatty acid as well as the alcohol, are integral constituents of the lipid region. We have therefore synthesized various 1,1-dideuterio alcohols (by  $\text{LiAlD}_4$  reduction) and also 10,10,10-trideuterio-decanoic acid (by Kolbe electrolysis)<sup>16</sup> and have used them as components in bilayer phases. Representative examples of dmr spectra obtained with nonoriented and oriented samples of such bilayers are shown in Figures 1 and 2, respectively.

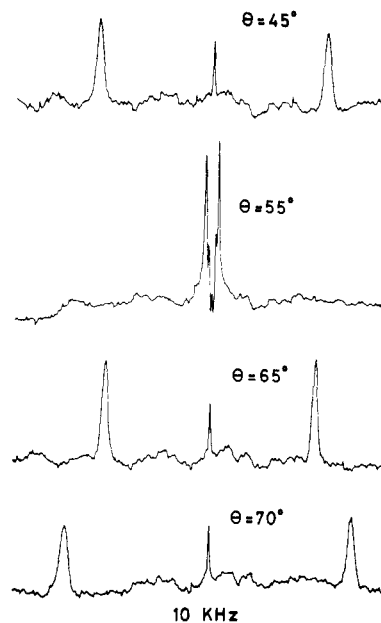
It should be noted that oriented bilayers yield more intense spectra than nonoriented systems, especially if the bilayer normal is oriented near the "magic angle" ( $\theta = 54.7^\circ$ ), where the dipolar couplings to the surrounding protons are drastically reduced and the lines sharpen. Compared to a powder pattern spectrum, the amount of liquid crystalline material needed for a reasonable signal can be reduced by a factor of 20 in the

(13) L. J. Burnett and B. H. Müller, *J. Chem. Phys.*, **55**, 5829 (1971).

(14) J. Seelig, H. Limacher, and P. Bader, *J. Amer. Chem. Soc.*, **94**, 6364 (1972).

(15) P. Ekwall, L. Mandell, and K. Fontell in "Liquid Crystals 2," Part II, G. H. Brown, Ed., Gordon and Breach, New York, N. Y., 1969, p 325.

(16) N. D. Nguyen, *Ark. Kemi*, **28**, 289 (1968).



**Figure 2.**  $^2\text{H}$  nmr spectrum of oriented bilayers. Same phase as in Figure 1. A total of approximately 20 mg of material was sandwiched between a stack of 20 glass plates of  $20 \times 6.6 \times 0.15\ \text{mm}$ . The package was placed in a 10-mm sample tube. Each spectrum results from 24,000 free induction decays (accumulation time 16 min), using  $90^\circ$  pulses of  $20\ \mu\text{sec}$  length.  $\theta$  denotes the angle between the magnetic field and the normal on the glass plates, which is also the normal on the oriented bilayers. Smaller and larger angles of  $\theta$  have also been measured. The lines broaden even further and a part of the spectrum is backfolded. From the angular dependence of the observed separations a maximum splitting of  $\Delta\nu(\theta = 0^\circ) = 56.6 \pm 0.6\ \text{kHz}$  is calculated. The sharp signal in the center of the spectra arises from isotropic motion of deuterium-labeled molecules. During the preparation of the oriented samples a small fraction of an isotropic phase seems to form. The integrated intensity is, however, less than 10 % of the total intensity.

oriented case. The quadrupole splittings of the randomly distributed and oriented samples as well as the chemical composition are summarized in Table I.

## Discussion

Equation 2 predicts the  $\Delta\nu_r$  should be equal to  $\Delta\nu(\theta = 90^\circ)$ . This is not completely borne out by the experiments. The quadrupole splitting  $\Delta\nu_r$  is always found to be smaller than  $\Delta\nu(\theta = 90^\circ)$  (cf. Table I). The small difference could be due to lateral diffusion of the lipid molecules. A similar effect has also been observed with spin labels in systems of extreme curvature.<sup>17</sup> Another possibility to explain the differences is to assume structural changes in oriented bilayers, which could be caused by shearing forces and surface effects. But as spin labeled fatty acids yield the same order parameter, independent of the type of macroscopic alignment, this explanation seems less likely.

Experimentally, only the absolute value of the quadrupole splitting is accessible (unless  $S_{\text{C-D}} > 0.5$ ). Under certain circumstances the sign can be deduced, however, from geometrical considerations. This is particularly simple in the case of lipids. The long molecular axis of a lipid molecule is essentially parallel to the axis of motional averaging (i.e., the bilayer normal), and the C-H bond of a methylene unit is oriented more or less perpendicular to this axis. Such

(17) J. Seelig and H. Limacher, *Mol. Cryst. Liquid Cryst.*, in press.

**Table I.** Quadrupole Splittings and Order Parameters of Deuterium Labels in Bilayer Systems

System	Chemical composition, <sup>a</sup> wt %			$\Delta\nu_{\tau}$ , kHz	$\Delta\nu$ ( $\theta = 90^\circ$ ), kHz	$S_{C-D}^b$	$S_{mol}$
Ia	Decanoic acid (sodium salt), 28	1,1-Dideuterio-decanol, 42	Water, 30	$27.0 \pm 0.2$	$30.7 \pm 0.3$	-0.241	$0.482 \pm 0.005$
Ib	10,10,10-Trideuterio-decanoic acid (sodium salt), 28	1-Decanol, 42	Water, 30	$6.4 \pm 0.2$	$6.7 \pm 0.3$	-0.053	$0.159 \pm 0.005$
II	Octanoic acid (sodium salt), 40	1,1-Dideuterio-butanol, 21	Water, 39	$19.0 \pm 0.2$	$23.9 \pm 0.3$	-0.187	$0.374 \pm 0.005$
III	Octanoic acid (sodium salt), 37	1,1-Dideuterio-hexanol, 25	Water, 38	$24.1 \pm 0.2$	$27.6 \pm 0.3$	-0.216	$0.432 \pm 0.005$
IV	Octanoic acid (sodium salt), 35	1,1-Dideuterio-octanol, 30	Water, 35	$24.2 \pm 0.2$	$28.3 \pm 0.3$	-0.222	$0.444 \pm 0.005$
V	Octanoic acid (sodium salt), 32	1,1-Dideuterio-decanol, 35	Water, 33	$25.2 \pm 0.2$	$28.7 \pm 0.3$	-0.225	$0.45 \pm 0.005$

<sup>a</sup> Systems II-V have the same molar composition (8.6 mol % sodium octanoate, 9.7 mol % 1,1-dideuterio alcohol, 81.7 mol % H<sub>2</sub>O). <sup>b</sup> Calculated from  $\Delta\nu$  ( $\theta = 90^\circ$ ).

an orientation requires negative order parameters, and this is the reason for the negative sign in the penultimate column of Table I.

Even more interesting than the order parameter of the C-D bond is the order parameter of the long molecular axis, or to be more precise, the average orientation of the chain segment to which the deuterium is attached. Let us define this segment direction as given by the normal on the plane spanned by the two C-D bonds of a methylene group. With this definition the segmental orientations will coincide with the long molecular axis if the chain is frozen in the all-trans conformation. Since the segment direction is perpendicular to the C-D bonds, its order parameter  $S_{mol}$  can be derived from  $S_{CD}$  by means of the following relation

$$S_{mol} = S_{CD}[(3 \cos^2 90^\circ - 1)/2]^{-1} = -2S_{CD} \quad (3)$$

This situation is slightly different for the terminal methyl group. Here the assumption is made that the characteristic segment axis is given by the direction of the terminal carbon-carbon bond, which leads to the following transformation.

$$S_{mol} = S_{CD}[(3 \cos^2 109.5^\circ - 1)/2]^{-1} \approx -3.00S_{CD} \quad (4)$$

The segmental order parameters calculated according to eq 3 and 4 are given in the last column of Table I.

The residual quadrupole splittings can be measured with high accuracy ( $\pm 0.3$  kHz). The experimental error in the determination of the order parameters is therefore reduced by a factor of 3-4 compared to the spin label method, a result which can be looked on even more favorably since it is obtained free from any structural perturbations. A preliminary quantitative comparison between the two techniques can be made for the sodium decanoate-decanol bilayer (system I). The order parameter of the terminal methyl group of the deuterated decanoic acid is  $S_{mol} = 0.159$ , while a steric acid spin label with the NO group attached at the corresponding position (C-10) yields  $S_{mol} = 0.23$ .<sup>14</sup> In the vicinity of the polar region  $S_{mol} = 0.482$  is found for the first chain segment of decanol. Unfortunately, no equivalent spin label can be synthesized, but an approximate comparison can be made with stearyl alcohol labeled at C-4, yielding  $S_{mol} = 0.46$ .<sup>14</sup> Even from this limited amount of data two conclusions can be drawn. (1) Both methods detect increased

motional freedom of the chain segments in the central part of the bilayer. (2) The order parameters determined by deuterium labels are smaller than the corresponding spin label results. Due to the larger cross section at the position of the spin label group the motional freedom of this part of the chain is restricted. The spin label results must therefore be considered as a first approximation, while certain quantitative aspects of the bilayer structure need to be reassessed. We are at present synthesizing appropriate deuterium labels.

The other quadrupole splittings reported in Table I (systems II-V) refer to the chain length dependence of the segmental order parameter. The position of the deuterium is left constant at the first carbon atom, but the length of the hydrocarbon chain increases. This leads to a simultaneous increase of the order parameter. The same behavior has also been found using spin labels,<sup>14</sup> but again the two methods show quantitative differences, and the deuterium results seem more reliable.

As far as the hydrophobic region is concerned the physical structure of the bilayers investigated here is very similar to that of phospholipid lamellae and biological bilayer membranes. The characteristic properties in common to all liquid crystalline bilayers are the anisotropic motion of the hydrocarbon chains and the flexibility gradient, while the chemical nature of the bilayer building stones seems to be reflected in the absolute values for the order parameters and correlation times. The deuterium label method should be especially suited for elucidating these differences with great precision.

So far we have established that order parameters in lipid bilayers can easily be measured by selective deuterium labeling, but we have completely neglected the dynamic aspects of the bilayer structure. The second question which we asked at the beginning is much harder to answer. <sup>1</sup>H and <sup>13</sup>C relaxation time measurements of lecithin dispersions are generally interpreted in terms of a more "fluid" or more isotropic bilayer interior.<sup>7-9,18-20</sup> But it is not clear as yet to which extent this increase of relaxation times is due to

(18) Y. K. Levine, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *Biochemistry*, **11**, 1416 (1972).

(19) A. G. Lee, N. J. M. Birdsall, Y. K. Levine, and J. C. Metcalfe, *Biochim. Biophys. Acta*, **255**, 43 (1972).

(20) A. F. Horwitz, W. J. Horsley, and M. P. Klein, *Proc. Nat. Acad. Sci. U. S.*, **69**, 590 (1972).

faster motions and to which extent it is only another expression for the increased randomness of the motion. Deuterium labeling may again be helpful in solving this problem. The relaxation times of a deuterium resonance line are determined essentially by quadrupole relaxation. The interpretation of relaxation times becomes thus much simpler than that of  $^1\text{H}$  or  $^{13}\text{C}$  nuclei.

During the preparation of this manuscript a brief report appeared on the intercalation of selectively deuterated lauric acid in lecithin bilayers.<sup>21</sup> The

authors observed a sharp deuterium signal in sonicated bilayer dispersions. No information about order parameters was deduced, but from the line width the authors could give an estimate of the correlation time.

**Acknowledgment.** We wish to thank Dr. A. F. Horwitz for critically reading the manuscript. This work was supported by the Swiss National Science Foundation under Grant 3.8620.72.

(21) H. Saito, S. Schreier-Muccillo, and I. C. P. Smith, *FEBS (Eur. Biochem. Soc.) Lett.*, **33**, 281 (1973).

## Cobalt-59 Nuclear Quadrupole and Nuclear Magnetic Resonance Spectra of Cobaloximes<sup>1</sup>

Robert A. LaRossa<sup>2</sup> and Theodore L. Brown\*

*Contribution from the School of Chemical Sciences and Materials Research Laboratory, University of Illinois, Urbana, Illinois 61801. Received September 26, 1973*

**Abstract:** Cobalt-59 nuclear quadrupole and nuclear magnetic resonance spectra are reported for a series of compounds  $\text{XCo}(\text{dh})_2\text{L}$ , where  $\text{dh}$  = dimethylglyoxime monoanion,  $\text{X}^- = \text{CH}_3^-$ ,  $\text{CHCl}_2^-$ ,  $\text{Cl}^-$ , and  $\text{Br}^-$ , and  $\text{L}$  = Lewis base. Chlorine-35 nqr signals were observed in a few compounds. Interpretation of the  $^{59}\text{Co}$  quadrupole coupling constants and asymmetry parameters in terms of bonding and electron distribution in the molecules requires an understanding of the orientation of and contributions to the electric field gradient tensor. By taking into account the known geometry of these complexes, a "donated charge" model is used to describe the changes in the quadrupole coupling constant and asymmetry parameter which result from changes in the interactions between the cobalt atom and the ligands. Taken in conjunction with the  $^{59}\text{Co}$  chemical shifts, the measured nqr parameters are interpreted in terms of the relative partial field gradient contributions of the axial and planar nitrogen ligands,  $[\text{X}]$  and  $[\text{N}]$ , respectively. Comparison of calculated  $eq_{zz}$  and  $\eta$  values with experimental values of  $e^2q_{zz}Q/h$  and  $\eta$  shows that the value of  $[\text{N}]$  depends on the nature of the axial ligands in such a manner that the total of the partial field gradient contributions from all the ligands about Co is approximately constant. The axial ligands are ordered in terms of their interaction with Co as measured by the ratio  $[\text{N}]/[\text{X}]$ .

**A** knowledge of the electronic charge distribution in molecules and ions is essential in attempts to understand bonding and the manner in which bonding is altered as a result of structural variations. Unfortunately, few experimental techniques provide observables which are directly related to the charge distribution.

Nuclear quadrupole resonance (nqr) spectroscopy provides a direct measure of the charge distribution around a quadrupolar nucleus in the electronic ground state of a molecule or ion through the interaction of the nuclear quadrupole moment ( $eQ$ ) with the electric field gradient (efg). The interaction is expressed in terms of the two independent components of the field gradient tensor, the quadrupole coupling constant,  $e^2q_{zz}Q/h$ , and asymmetry parameter

$$\eta = \frac{q_{zz} - q_{yy}}{q_{zz}} \quad (1)$$

where  $q_{xx}$ ,  $q_{yy}$ , and  $q_{zz}$  are the principal components of the efg tensor. The asymmetry parameter reflects the extent of deviation of the tensor from axial symmetry.<sup>3-5</sup>

(1) This research was supported by the National Science Foundation through Research Contract GH-33634 and Research Grant GP-30256X.

(2) University of Illinois Fellow, 1971-1972.

(3) T. P. Das and E. L. Hahn, "Nuclear Quadrupole Resonance Spectroscopy," Academic Press, New York, N. Y., 1958.

In the application of nqr spectroscopy to the nucleus of a multiply connected atom in a complex compound, the interpretation of the observed quadrupole coupling constant and asymmetry parameter may become quite difficult. If all the information from nqr spectroscopy is to be utilized effectively, the geometrical parameters must be known. Bis(dimethylglyoximate)cobalt(III) compounds (cobaloximes) are a series in which the geometry about the cobalt atom is well known and nearly invariant upon changes in ligation along the vertical axis.<sup>6-8</sup>

These compounds provide not only an interesting test of the utility of the nqr technique in the study of complex molecules but also provide a good basis for the evaluation of currently popular models which describe the origin of electric field gradients in semiempirical

(4) E. A. C. Lucken, "Nuclear Quadrupole Coupling Constants," Academic Press, New York, N. Y., 1969.

(5) E. Schempp and P. J. Bray in "Physical Chemistry. An Advanced Treatise," Vol. IV, D. Henderson, Ed., Academic Press, New York, N. Y., 1970, Chapter 11.

(6) J. S. Swanson, Ph.D. Thesis, University of Illinois, Urbana, Ill., 1971.

(7) (a) P. G. Lenhart, *Chem. Commun.*, 980 (1967); (b) W. W. Adams and P. G. Lenhart, *Acta Crystallogr., Sect. B*, **29**, 2412 (1973).

(8) K. S. Viswanathan and N. R. Kuncher, *Acta Crystallogr.*, **14**, 675 (1961). The structure reported,  $[(\text{NH}_3)_2\text{Co}(\text{dh})_2]\text{NO}_3$ , was not determined with high accuracy, and the reported N-Co-N angle of  $78^\circ$  is subject to considerable uncertainty.