### Supporting Information

| Brain | Clinical diagnosis | Post-mortem delay (days) | Fixative type | Time in fixative before scanning (days) |
|-------|--------------------|--------------------------|---------------|-----------------------------------------|
| 1     | Control            | 3                        | 10% NBF       | 115                                     |
| 2     | Control            | 3                        | 10% NBF       | 45                                      |
| 3     | Control            | 3                        | 10% NBF       | 48                                      |
| 4     | ALS                | 1                        | 10% NBF       | 35                                      |
| 5     | ALS                | 2                        | 10% NBF       | 87                                      |
| 6     | ALS                | 3                        | 10% NBF       | 94                                      |
| 7     | ALS                | 2                        | 10% NBF       | 139                                     |
| 8     | ALS                | 4                        | 10% Formalin  | 178                                     |
| 9     | ALS                | 3                        | 10% Formalin  | 114                                     |
| 10    | ALS                | 7                        | 10% Formalin  | 137                                     |
| 11    | ALS                | 4                        | 10% Formalin  | 94                                      |
| 12    | ALS                | 3                        | 10% Formalin  | 158                                     |
| 13    | ALS                | Data Unavailable          | 10% Formalin  | 96                                      |
| 14    | ALS + FTD          | 2                        | 10% Formalin  | 283                                     |

Table S1: Characteristics of each brain used in this study. Here ALS – Amyotrophic Lateral Sclerosis, FTD – Frontotemporal Dementia, NBF – Neutral Buffered Formalin.

| Brain | 1-3, 7 | 8 | 9-12 | 4,13 | 5-6,14 |
|-------|--------|---|------|------|--------|
| No. echoes | 6 | 6 | 6 | 6 | 6 |
| TE (ms) | 13, 25, 38, 50, 63, 76 | 11, 23, 34, 46, 57, 69 | 11, 23, 34, 46, 57, 69 | 11, 23, 34, 46, 57, 69 | 10, 20, 30, 40, 50, 60 |
| TR (ms) | 1000 | 1000 | 1000 | 1000 | 1000 |
| Resolution (mm³) | 0.9 x 0.9 | 0.8 x 0.8 | 0.8 x 0.8 | 0.65 x 0.65 | 1.0 x 1.0 |
| Bandwidth (Hz/pixel) | 166 | 195 | 195 | 163 | 199 |
| Turbo factor | 6 | 6 | 6 | 6 | 6 |
| Time per TE (minutes) | 36 | 26 | 16 | 18 | 24 |
| Slice resolution (%) | 100 | 100 | 50 | 50 | 100 |

Table S2: Acquisition parameters for the TSE scans used in this study. Details of individual brains provided in Table S1.
Estimating $T_2$ using an Extended Phase Graph (EPG) model

The TSE sequence is characterised by a train of RF pulses ($90^\circ$-$180^\circ$-$180^\circ$-$180^\circ$…), where the signal is sampled after each $180^\circ$ pulse. Assuming a single tissue compartment and a perfect $180^\circ$ pulse, the TSE signal evolves via a characteristic mono-exponential decay (Fig. S1a – red line). At 7T, $B_1$ inhomogeneity produces a spatially varying flip angle across the brain (Fig. S1b), leading to refocusing pulses that vary from $180^\circ$. Under these conditions, the signal evolution can deviate substantially from a mono-exponential signal model (Fig. S1a – purple, blue and green lines). Fitting a mono-exponential signal model to these data will lead to estimates of $T_2$ that strongly depend on the $B_1$ profile (Fig. S1c).

Extended Phase Graphs (EPG) provide a more accurate description of signal evolution under a variety of MRI sequences and conditions (1–3). In this work, we use an EPG framework to describe the signal evolution of the TSE sequence under a refocusing pulse of flip angle $\alpha$. Our approach fits the measured TSE signal to estimate the voxelwise $T_2$ and $\alpha$ (via estimation of $B_1$). Fitting with an EPG model is shown to reduce the bias on $T_2$ estimates in areas of low $B_1$, leading to more homogeneous $T_2$ maps across the brain (Fig. S1d).

To achieve this, we performed a two-step fitting approach, where we first fit the TSE signal to an EPG model to estimate $T_2$ and $B_1$ across the brain. It was found that in regions of very low $B_1$, sharp discontinuities were observed in the $B_1$ maps. Given that $B_1$ is expected to smoothly vary across the brain, we subsequently smoothed the $B_1$ map and repeated the fitting for $T_2$, keeping $B_1$ as a fixed parameter.

**Step one:**

For each postmortem brain we simulated the TSE sequence (parameters provided in Table S2) under an EPG framework, obtaining voxelwise estimates of $B_1$ and $T_2$ by minimising:

$$\min_{B_1,T_2} \| TSE_{\text{exp}}(x,y,z, \text{TE}_{1:6}) - TSE_{\text{sim}}(B_1,T_2,T_1,\text{TE}_{1:6}) \|^2_2,$$

where $TSE_{\text{exp}}$ is the experimental TSE data over all six echoes ($\text{TE}_{1:6}$) and $TSE_{\text{sim}}$ is the simulated TSE signal using the EPG framework given a value of $B_1$, $T_2$, $T_1$, and the echo times. To avoid fitting for the signal amplitude ($S_0$), the experimental data and simulated signal were normalised (e.g. division by $\left( \sum_{j=1}^6 TSE_j^2 \right)^{0.5}$). In a series of evaluations, $T_1$ was found to have very little effect on our $T_2$ estimates, after which it was set equal to a fixed
constant (450 ms - the approximate value of measured $T_1$ in our postmortem datasets).

Fitting was performed in MATLAB (version 2019b, The MathWorks, Inc., Natick, MA) based on code from (3). Eq. [S1] was minimised using *lsqnonlin*.

Figures S2a and b show the resulting $B_1$ and $T_2$ maps for a single postmortem brain fit with this approach. The spatial inhomogeneity across the $T_2$ map is substantially reduced in comparison to the mono-exponential fit (Fig. S1c). However, $B_1$ maps are expected to vary smoothly across the brain and Fig. S2a reveals changes in $B_1$ that depend on the tissue contrast (e.g. blue arrow), in addition to underestimation of $B_1$ close to the brain boundary.

Figure S1: Motivation for the EPG framework. When the refocusing angle = 180° (a – red line), the TSE signal evolves via a mono-exponential decay. However, at different flip angles the signal evolution can deviate substantially from a mono-exponential signal model (a – purple, blue and green lines). At 7T, brain samples experience $B_1$ inhomogeneity, leading to a spatially varying flip angle across the brain (b). Fitting these data with a mono-exponential model leads to a $T_2$ map that depends strongly on the $B_1$ profile (c). An EPG framework is able to account for the spatially varying flip angle across the brain, leading to $T_2$ maps with more homogeneous contrast (d). (a) simulated using an EPG framework (TE = 10 – 60 ms with a 10 ms echo spacing, $T_2 = 30$ ms), with resulting curves normalised to the signal at TE = 10 ms to aid visualisation of the deviation from a mono-exponential decay. Note that there is a degeneracy when $\alpha > 180^\circ$, where flip angle $\alpha$ produces the same signal evolution as $360^\circ - \alpha$. (c) - Mono-exponential (d) - EPG

(a) - TSE signal vs flip angle

(b) - Flip angle

(c) - Mono-exponential

(d) - EPG
Figure S2: T<sub>2</sub> estimates with EPG and smoothing B<sub>1</sub>. Step 1 - Our EPG model estimates B<sub>1</sub> maps with decreasing B<sub>1</sub> as the brain boundary is approached (a), in addition to T<sub>2</sub> maps that do not have a strong dependence on the B<sub>1</sub> profile (b). The B<sub>1</sub> profile is expected to be smoothly varying across the brain. However, anatomical contrast is visible within the B<sub>1</sub> map (a – blue arrow), in addition to sharp discontinuities in areas close to the brain boundary (a - green arrows), regions associated with low SNR. This leads to artefacts and subtle contrast changes in the resulting T<sub>2</sub> map (b – blue and green arrows). Step 2 - By smoothing the B<sub>1</sub> maps with a polynomial filter (c) and fixing B<sub>1</sub> to the resulting map in a second stage estimate of T<sub>2</sub>, we obtain consistent T<sub>2</sub> estimates across the brain (d). Here the B<sub>1</sub> maps (a and c) are displayed between 0 and 1, with the T<sub>2</sub> maps (b and d) displayed between 0 and 60 ms. N.B (d) does not include the regularisation step (Eq. [S3]).

(e.g. green arrows). This leads to clear artefacts in the resulting T<sub>2</sub> maps (Fig. S2b), most apparent in regions where B<sub>1</sub> is underestimated (green arrows).

**Step two:**
To ensure spatial smoothness of B<sub>1</sub>, the B<sub>1</sub> maps from step 1 were subsequently filtered using a local 3D polynomial filter (order = 2, kernel volume = 10x10x10 mm<sup>3</sup>), with voxels weighted by the inverse of the standard error on the B<sub>1</sub> estimates. Figure S2c displays an example B<sub>1</sub> map after filtering, revealing a smoothly varying B<sub>1</sub> profile across the entire brain.
\[ \min_{T_2} \left\| TSE_{exp}(x, y, z, T, 1, 1.6) - TSE_{sim}(B_1, x, y, z, T_2, T_1, T, 1.6) \right\|^2_2, \]

where \( B_1 \) was fixed to the value of the smoothed \( B_1 \) map. Figure S2d displays the resulting \( T_2 \) map, where the most notable change is in regions of the brain where the \( B_1 \) was previously underestimated (Fig. 2d green arrows) – in these regions the \( T_2 \) estimates now match those of the surrounding tissue.

In regions very close to the brain boundary (characterised by very low \( B_1 \) and low SNR), in some brains the \( T_2 \) estimates were found to be highly sensitive to noise, leading to spurious values (Fig. S3a). To correct for this, regularisation was added to Eq. [S2] as follows:

\[ \min_{T_2} \left\| W \cdot [TSE_{exp}(x, y, z, T, 1, 1.6) - TSE_{sim}(B_1, x, y, z, T_2, T_1, T, 1.6)] - \lambda \right\|_2^2, \]

where \( \lambda \) is a regularisation constant (\( \lambda = 2 \)), \( T_{2,med} \) is the median value of \( T_2 \) across the entire brain from the first step fitting and \( W \) is a scalar weight \( \left( \sum_{j=1}^{6} TSE_{exp, j}^2(x, y, z) \right)^{0.5} \).

Figure S3b displays a \( T_2 \) map with the addition of regularisation. Differences between the two maps (Fig. S3c) are restricted to these areas of very low \( B_1 \) and SNR. Figure 5 (Main Text) displays the \( T_2 \) maps fit using this approach for all 14 brains used in our study.
Figure S3: Addition of regularisation for our T<sub>2</sub> estimates. In areas of low B<sub>1</sub> in close proximity to the brain boundary, the TSE data had very low SNR. In some of our T<sub>2</sub> maps, this lead to spurious T<sub>2</sub> estimates (visible in (a)). The addition of regularisation (b) via fitting with Eq. [S3] bought the T<sub>2</sub> values within these regions into agreement with the surrounding tissue. Within other areas of tissue, the regularisation led to negligible changes in T<sub>2</sub> (c).
Correcting for spuriously high diffusion coefficients

It is not possible to determine diffusion coefficients in voxels with unreliable signal estimates. In this study, unreliable signal estimates arose (1) in voxels in very close proximity to remaining air bubbles, (2) in regions of extremely low SNR, and (3) due to imperfect masking (resulting in a small number of non-brain tissue voxels remaining in the brain mask, e.g. small quantities of signal-producing formalin remaining in brain sulci and ventricles). In these voxels, diffusion coefficients manifested as spuriously high values on our resulting diffusion tensor estimates.

The number of unreliable voxels was small, with an average of 1.9 ± 2.1% of voxels / brain requiring correction, below 0.6% in 7 of the 14 brains. However, it is critical to correct for these voxels for two reasons. First, an accurate brain shape is important for modelling fixative dynamics - removal of these voxels would lead to a change the brain shape, or holes in the brain. Second, spuriously high diffusion coefficients can lead to artefacts in the resulting fixative concentration maps (Fig. S4). These artefacts arise when a large concentration gradient across neighbouring voxels induces a change in concentration > 1 in a single iteration of the fixative dynamics simulation (Main Text Appendix Eq. [A1]), resulting in negative concentration values. One approach to prevent these artefacts is to reduce the time step per iteration (τ in Main Text Appendix Eq. [A1]). However, this can considerably increase the time required to perform the fixative simulations. Notably, even a single voxel can give rise to this artefact, motivating our use of a threshold to remove the spuriously high diffusion coefficients.
Figure S4: Artefacts arising in fixative concentration simulations due to spuriously high diffusion coefficients. (a) displays an axial slice of the principal eigenvalue (L1) map of a single post-mortem brain, with (b) displaying a mask of voxels containing the spuriously high diffusion coefficients (>1 \times 10^{-3} \text{ mm}^2/\text{s}). (c) and (d) display the concentration distribution map after two days of fixative outflow as described in the Methods (Main Text) without (c) and with (d) correction for high diffusion coefficients. Failure to perform the correction leads to artefacts in the resulting concentration map (c), most apparent in the regions highlighted by the orange arrows. Correction of these voxels (d) results in a smooth concentration map. Mean diffusivity map (a) displayed between 0 and 5 \times 10^{-3} \text{ mm}^2/\text{s}, concentration distributions (c & d) displayed between 0 and 1.
Additional Supporting Figures and Tables

Figure S5: Distribution of white / grey matter. For all three models, white and grey matter are not evenly distributed with respect to distance from the nearest surface or fixative concentration. Plots generated over all 14 brains, with error bars representing the standard deviation between brains. Voxels < 2 mm from the nearest surface were not included in our analysis and are therefore not displayed here.

Figure S6: Fixative concentration vs PLP SAF across M1, ACC and V2. For brains fixed with 10% NBF, a small (but significant) correlation was found between the concentration of fixative and the PLP SAF, where there is a notable distinctions in the fixative concentration and PLP SAF in individual regions (e.g. ACC and M1 – hand region). For Brains fixed with 10% formalin, no such relationship was observed.
Figure S7: Fixative concentration vs ferritin SAF across M1, ACC and V2. No significant correlation was observed between fixative concentration and ferritin SAF for brains fixed with 10% NBF or 10% formalin. Note that as the ferritin SAFs were normalised for the two batches, the SAF values can be positive & negative and are not restricted to a range between 0 and 1. As the ACC and V2 regions were included in both batches, the ferritin SAFs were averaged prior to analysis & plotting.
Figure S8: $T_2$ vs $B_1$ over white and grey matter for all post-mortem brains fixed with 10% NBF and 10% formalin. The corrections presented in this work assume that presence of fixative in tissue is the source of variation in $T_2$ across the brains. However, the predicted patterns of fixative are similar to the spatial pattern of $B_1$. Here, we consider how much of the variation in $T_2$ may be explained by the $B_1$ spatial profile. A dependency is observed vs $B_1$ in our post-mortem cohort (a), with a stronger effect in brains fixed with 10% NBF (left) vs 10% formalin (right), consistent with the other three models investigated in this study (Main Text Figs. 7 and 8). However, regressing out the influence of $B_1$ using Eq. [6] (b) leads to a higher remaining inhomogeneity as shown in Table S3 vs the D2S, KL and KT models for the brains fixed with 10% NBF (Main Text Table 1), and similar performance (no change) for brains fixed with 10% formalin. Results displayed as the mean ± standard deviation across brains.
(a) **10% NBF**

| Tissue Type         | Uncorrected | $B_1$ correction |
|---------------------|-------------|------------------|
| White Matter        | 2.80 ± 0.41 | 2.56 ± 0.34      |
|                     |             | (0.26)           |
| Grey Matter         | 6.11 ± 0.77 | 5.79 ± 0.70      |
|                     |             | (0.43)           |

(b) **10% Formalin**

| Tissue Type         | Uncorrected | $B_1$ correction |
|---------------------|-------------|------------------|
| White Matter        | 1.46 ± 0.23 | 1.45 ± 0.21      |
|                     |             | (0.89)           |
| Grey Matter         | 2.45 ± 0.32 | 2.45 ± 0.32      |
|                     |             | (0.99)           |

**Table S3:** Inhomogeneity over white and grey matter for brains fixed with 10% NBF and 10% formalin – correction with $B_1$. Correction with $B_1$ gave rise to a reduction in inhomogeneity across both grey and white matter for brains fixed with 10% NBF. However, this reduction in inhomogeneity did not reach significance, and was a smaller improvement vs the D2S, KI and KT models (Main Text Table 1). Little change was found in brains fixed with 10% formalin, consistent with the corrections using the D2S, KI and KT models (Main Text Table 1). $p$-values comparing the change in inhomogeneity for the $B_1$ correction displayed in brackets.
Figure S9: \( T_2 \) vs concentration for the KI model incorporating mean diffusivity maps. The KT model presented in this work incorporates both diffusion anisotropy and voxel-specific diffusion coefficients to simulate fixative dynamics. Here, we consider how much of the variation in \( T_2 \) can be explained by the voxel-specific diffusion coefficients alone. Simulations using the KI model incorporating voxel-wise mean diffusivity maps yields very similar \( T_2 \)-concentration distributions to the KT model (Main Text Figs. 7 and 8). Regressing out the influence of the KI model incorporating these mean diffusivity maps using Eq. [6] (b) leads to a marginally higher remaining inhomogeneity (Table S4) compared to the KT model for brains fixed with 10% NBF (Main Text Table 1), and similar performance (no change) for brains fixed with 10% formalin. This indicates that voxel-wise variations in diffusivity are the predominant driver of fixative dynamics in the KT model, with diffusion anisotropy less crucial for reducing \( T_2 \) inhomogeneity. Results displayed as the mean ± standard deviation across brains.
Table S4: Inhomogeneity over white and grey matter for brains fixed with 10% NBF and 10% formalin – correction with the KI model incorporating mean diffusivity maps. Correction with the KI model incorporating mean diffusivity maps led to inhomogeneity estimates similar to the KT model for brains fixed with both 10% NBF and 10% formalin (Main Text Table 1). p-values comparing the change in inhomogeneity for the model correction displayed in brackets.

(a) 10% NBF

| Tissue Type    | Uncorrected | KI Mean Diffusivity correction |
|----------------|-------------|-------------------------------|
| White Matter   | 2.80 ± 0.41 | 2.17 ± 0.16 (0.0025)          |
| Grey Matter    | 6.11 ± 0.77 | 5.37 ± 0.50 (0.055)           |

(b) 10% Formalin

| Tissue Type    | Uncorrected | KI Mean Diffusivity correction |
|----------------|-------------|-------------------------------|
| White Matter   | 1.46 ± 0.23 | 1.44 ± 0.21 (0.86)            |
| Grey Matter    | 2.45 ± 0.32 | 2.47 ± 0.33 (0.91)            |
Figure S10: $T_2$ vs concentration for the KI model incorporating white and grey matter diffusivity estimates.

The KI model presented in this work simulates fixative dynamics utilising a single, global scalar of diffusivity, set as the average mean diffusivity over the entire brain. Here we repeat the simulations setting the global scalar as the average mean diffusivity over (a) white matter and (b) grey matter. Regressing out the influence of the KI model using Eq. [6] with the average over white matter (a) leads to an improved homogeneity for grey matter for NBF brains, but a considerable reduction in white matter homogeneity verses the original KI model (confirmed in Table S5). Correction with the average over grey matter for NBF brains (b) leads to a slight reduction in homogeneity over grey matter verses the original KI model. Similar performance (no change) was found for brains fixed with 10% formalin. Neither model demonstrated improved performance over the KT model. Results displayed as the mean ± standard deviation across brains.
(a) **10% NBF**

| Tissue Type | Uncorrected | $K_I$ – white matter correction | $K_I$ – grey matter correction |
|-------------|-------------|--------------------------------|--------------------------------|
| White Matter | 2.80 ± 0.41 | 2.51 ± 0.34 | 2.25 ± 0.17 |
| Grey Matter  | 6.11 ± 0.77 | 5.39 ± 0.52 | 5.47 ± 0.53 |

(b) **10% Formalin**

| Tissue Type | Uncorrected | $K_I$ – white matter correction | $K_I$ – white matter correction |
|-------------|-------------|--------------------------------|--------------------------------|
| White Matter | 1.46 ± 0.23 | 1.48 ± 0.22 | 1.44 ± 0.22 |
| Grey Matter  | 2.45 ± 0.32 | 2.28 ± 0.20 | 2.46 ± 0.33 |

Table S5: **Inhomogeneity over white and grey matter for brains fixed with 10% NBF and 10% formalin – correction with the $K_I$ model incorporating white and grey matter diffusivity estimates.** Correction with the $K_I$ model incorporating the average mean diffusivity over white matter (middle column) and grey matter (right column). Similar performance was found for brains fixed with 10% formalin versus the $K_I$ and $K_T$ model (Main Text Table 1). However, the white matter diffusivity leads to an improved homogeneity for grey matter for NBF brains, but a considerable reduction in white matter homogeneity versus the original $K_I$ model. Correction with the average over grey matter for NBF brains leads to a slight reduction in homogeneity over grey matter versus the original $K_I$ model. Neither model demonstrated improved performance over the $K_T$ model. *p*-values comparing the change in inhomogeneity for the model correction displayed in brackets.
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