# $T_2$-MRI mapping as a minimally-invasive correlate of central nervous system (CNS) toxicity in a cuprizone-model: A biomarker study

Syed Z. Imam[1], Kelly Ball[1], Ramesh C. Kovi[7], Christopher J. Somps[7], Natalya Sadovova[1], James Raymick[1], Bonnie Robinson[1], Joseph P. Hanig[4], Jennifer Beck Pierson[2], Ruth Roberts[3], Michael Aschner[5], William Slikker Jr.[6], Tucker A. Patterson[1], Serguei Liachenko[1]

[1]US FDA, NCTR, Jefferson, AR 72079, USA
[2]HESI, 740 15th St, NW, Suite 600, Washington, DC 20005, USA
[3]University of Birmingham, Birmingham B15 2TT, UK
[4]US FDA CDER, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA
[5]Albert Einstein College of Medicine, Forchheimer 209, 1300 Morris Park Ave, Bronx, NY 10461, USA
[6]Retired, US FDA, NCTR, Jefferson, AR 72079, USA
[7]Pfizer, 558 Eastern Point Rd, Groton, Connecticut 06340 and Cambridge, MA 02139, USA

## Background

Neurotoxicity has been linked to exposure to a number of drugs and chemicals, yet efficient, predictive, and minimally-invasive methods to detect neuropathological effects are not available in non-clinical assessments. Previously, we have shown significant $T_2$-MRI changes in a rat model of trimethyltin neurotoxicity that correlates with CNS neurotoxicity and pathology. Here, our main objective is to identify possible changes in $T_2$-MRI relaxation that predicts myelin-specific neurotoxicity resulting from exposure to a known neurotoxic agent, cuprizone, by correlating $T_2$-MRI relaxation with neuropathological endpoints. Adult rats (3-months old) were exposed to a daily dose of cuprizone (600 mg/kg p.o.) or corn oil for 4 weeks. Prior to treatment, animals underwent baseline MRI scans. Additional scans were done weekly after beginning exposure to cuprizone. At the end of 4 weeks, a final MRI was completed, and fluids and tissue samples were collected. Significant $T_2$-MRI relaxation increase was observed in the deep cerebellar nuclei in cuprizone-treated rats compared to controls. Histopathological analysis also revealed moderate demyelination in deep cerebellar nuclei. Our data demonstrate that MRI-based endpoints may be used as a robust minimally-invasive neurotoxicity biomarker in a myelin-specific neurotoxicity model.

## Methods

Animals: Adult Sprague Dawley rats were housed 2-per cage (12-hour light dark cycle) and given ad-libitum access to food and water.

Experimental treatment: Animals (N=8; 4 males and 4 females) were exposed to a single daily dose of cuprizone or vehicle (corn oil). Cuprizone was given at 600 mg/kg (3 ml/kg) bwt, p.o. for 4 weeks. Prior to treatment, animals underwent MRI scans to establish a baseline.

Magnetic Resonance Imaging: MRI was performed using a Bruker BioSpec 4.7T/40 system with a 38 mm litz-cage transmit/receive quadrature RF coil. Animals were anesthetized using isoflurane (3% induction in the chamber, 1-2% maintenance in 1L/min oxygen via nose cone) and body temperature was maintained at 37.0 ± 0.5 °C for the duration of scans. Whole brain $T_2$ relaxation mapping was performed using a multi-echo, fast spin echo sequence (8 echoes with 12 ms spacing, TR = 6 s, NA = 2). Three orthogonal scans were combined using wavelet transformation into single isotropic volume (MTX = 180 x 180 x 180, 0.2 mm/pixel) and $T_2$ maps were produced by exponential fitting of echo images pixel-by-pixel. Regions of interest (ROI) were manually drawn on deep cerebellar nuclei to calculate averaged $T_2$ values in those areas. Statistical analysis of $T_2$ data was performed using repeated measures ANOVA.

Pathology: At the end of the study, the following organs were collected for microscopic evaluation: Brain, spinal cord, peripheral nerves (sciatic and median), adrenal glands, thymus, liver, kidney, and heart. Samples were fixed in 10% neutral buffered formalin, trimmed and embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E), with selected brain sections stained with Kluver Barrera (Luxol fast blue) stain. The H&E and KB-stained slides were evaluated by a board-certified veterinary pathologist. Microscopic findings were graded on the following severity scale: 0 = within normal limits, 1 = minimal, 2 = mild, and 3 = moderate, 4 = marked, and 5 = severe.

## Objective

To identify minimally-invasive biomarkers that predict neurotoxicity resulting from exposure to a known neurotoxic agent, cuprizone, by correlating imaging and neuropathological endpoints. An additional goal was to develop a suitable model of myelin degeneration to represent a progressive model of rare neurological disorders.

## Cuprizone Treatment Results in Significant $T_2$-Relaxation in Deep Cerebellar Nuclei of Adult Rats

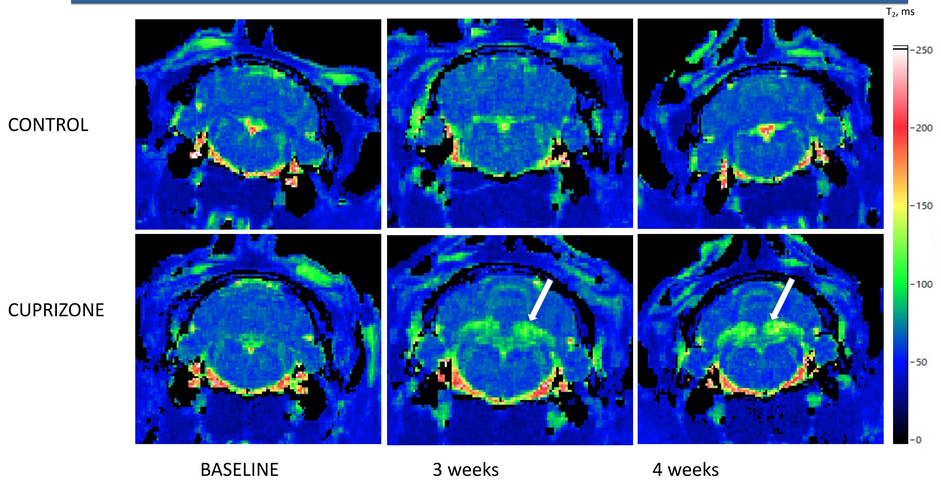


Figure 1. An example of a $T_2$ relaxation map of control (top panel) and cuprizone-treated (bottom panel) animals at baseline and 3 or 4 weeks shows a $T_2$ increase in the deep cerebellar nuclei (indicated by white arrow). Table 1 below represents averaged $T_2$ values (in ms) in deep cerebellar nuclei in control and cuprizone-treated animals at baseline and at 2, 3 and 4 weeks of treatment. P values represent the statistical significance between control and cuprizone groups. Data are means ± S.E.Ms.

Table 1. Averaged $T_2$ values (ms) in Deep Cerebellar Nuclei

| | Baseline | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| **CONTROL** | 63.16 ± 1.44 | 64.10 ± 4.43 | 64.67 ± 2.34 | 64.44 ± 2.64 |
| **CUPRIZONE** | 65.41 ± 3.11 | 66.88 ± 1.67 | 84.31 ± 2.04*# | 95.97 ± 4.64*# |

* - statistical difference between control and cuprizone groups, P < 0.001
# - statistical difference from baseline, P < 0.001

## Cuprizone Treatment Results in Significant Demyelination in Deep Cerebellar Nuclei of Adult Rats

**Table 2. Group incidence and severity of microscopic findings in rats**

| Groups | Vehicle (Corn Oil) | Cuprizone |
|---|---|---|
| **Dose (vg/kg)** | **1 ml/kg** | **600 mg/kg** |
| **N per group (Gender)** | **8 (4M+4F)** | **5 (3M+2F)** |
| **Brain** | | |
| Deep Cerebellar Nuclei , Demyelination/Vacuolation | **0** | **5** |
| Minimal (Grade 1) | 0 | 1 |
| Mild (Grade 2) | 0 | 3 |
| Moderate (Grade 3) | 0 | 1 |
| Corpus Callosum & Internal Capsule, Vacuolations | **0** | **4** |
| Minimal (Grade 1) | 0 | 4 |
| **Peripheral Nerve, Sciatic** | | |
| Nerve Fiber Degeneration | **0** | **5** |
| Minimal (Grade 1) | 0 | 1 |
| Mild (Grade 2) | 0 | 2 |
| Moderate (Grade 3) | 0 | 2 |

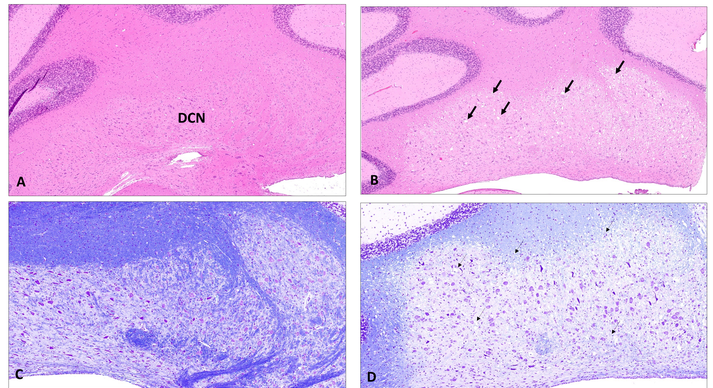


**Figure 2. Microscopic findings in rats dosed with cuprizone at 600 mg/kg.** A: H&E stain and C: Kluver Barrera (Luxol Fast Blue special stain for myelin) of deep cerebellar nuclei (DCN) from a vehicle control group showing normal myelination at the gray-white matter junction and within the DCN; B: H&E stain and D: Kluver Barrera (Luxol Fast Blue special stain for myelin) of DCN from a cuprizone-treated group showing moderate demyelination at the gray-white matter junction and within the DCN with vacuolations and loss of myelin staining (arrows).

## Results & Conclusion

- Cuprizone treatment resulted in significant changes in $T_2$-relaxation in deep cerebellar nuclei of adult rats, an area which is frequently targeted after cuprizone exposure.
- Although a trend in $T_2$-relaxation was observed starting week 2, significant changes were seen from week 3 onward over the course of a 4-week oral treatment of cuprizone.
- Neuropathological analysis confirmed cuprizone-mediated demyelination at the gray-white matter junction and within deep cerebellar nuclei along with vacuolation and loss of myelin staining. Additionally, minimal vacuolation was also observed within corpus callosum and internal capsule white matter tracts, a secondary target area of cuprizone treatment.
- Mild to moderate sciatic nerve fiber degeneration was also observed after cuprizone exposure.
- No significant histopathological changes were observed in non-CNS tissues.

Our findings indicate a novel use of MRI $T_2$-relaxation as a useful minimally-invasive tool for predicting myelin degeneration in a rodent oral administration model of cuprizone-induced neurotoxicity. Plasma, serum, and urine samples are being further analyzed for possible fluidic biomarkers of cuprizone-induced neurotoxicity. Furthermore, this animal model might serve as a suitable pre-clinical model of rare myelin-degenerative CNS disorders.

Syed.Imam@fda.hhs.gov
www.fda.gov