Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

MP101/MP201

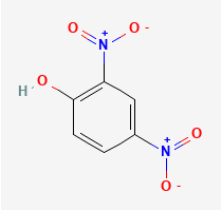
Evidence Summary

MP101 and/or MP201 have shown benefits in rodent models of AD, PD, MS, TBI, and others. DNP also increased longevity in mice. No clinical trials of MP101 or MP201 have been completed to date.

Neuroprotective Benefit: MP101/MP201 have shown neuroprotective benefits in rodent models of Alzheimer's, Parkinson's, multiple sclerosis, and brain injury. No human studies of MP101 or MP201 in neurodiseases have been completed to date.

Aging and related health concerns: DNP was used as a weight loss agent until it was banned due to toxicity. In rodents, doses of DNP that are up to 100-fold lower increased longevity, and improved metabolic indices, hearing loss, and nerve injury.

Safety: High doses of DNP were used for weight loss, which caused dangerous adverse events including death. Doses of MP101 that show protective benefits in mouse models are ~10-100 times lower. No clinical trials of MP101/201 have been completed to date.

Availability: in clinical development	**Dose**: Dosage has not been established. MP101 and MP201 are oral drugs.	**Chemical formula:** $C_6H_4N_2O_5$ for MP101 **MW**: 184.11 for MP101  Source: PubChem
Half-life: not documented	**BBB**: MP101 is penetrant in mice.	
Clinical trials: No clinical trials have been completed. A phase I/IIa study is ongoing.	**Observational studies**: N/A	

What is it?

Hormesis is defined by the phenomenon that low to moderate levels of stress or damage results in a protective adaptive response. An example of this is seen with intermittent metabolic challenges, including intermittent fasting or vigorous physical exercise, which can bolster resistance to metabolic, oxidative, and excitotoxic stress, leading to neuroprotective effects (Mattson, 2012; Geisler et al., 2017).

MP101 (2,4-dinitrophenol, or DNP) is a mitochondrial uncoupling agent that causes protons to leak across the inner mitochondrial membrane and the protons are lost as heat, instead of returning through the ATPase channel to generate ATP (reviewed in Geisler et al., 2017). Mitochondrial uncoupling leads to a reduction in mitochondrial membrane potential, decreased ATP production, and a transient increase in intracellular calcium levels. Mild uncoupling triggers an adaptive bioenergetic stress response, including the activation of kinases and transcription factors such as CREB, PGC-1α, and NF-kB that, in turn, induce the expression of genes that enhance stress resistance and neuroplasticity (e.g., immediate early gene products Fos and Arc, the neurotrophic factor BDNF, the antioxidant enzyme SOD2, the regulator of mitochondrial biogenesis TFAM, and an inhibitor of the mTOR pathway, TSC2). Mild mitochondrial uncoupling can also enhance respiratory rates by reducing the formation of superoxide radicals and preventing the formation of mitochondrial reactive oxygen species.

MP201 is a prodrug of MP101 with a carbon linker displacing the hydrogen on the hydroxyl group, making it inactive (Geisler, 2019). Upon oral administration, MP201 enters the portal vein where the carbon linker is cleaved off by enzymes and MP101 is released in its active form.

MP101 is under clinical development by Mitochon Pharmaceuticals for the treatment of amyotrophic lateral sclerosis (ALS), Huntington's disease, and multiple sclerosis (Mitochon pipeline). In 2019, Mitochon Pharmaceuticals was awarded Orphan Drug Designation by the FDA for MP101 for treating Huntington's disease (Mitochon press release, March 5, 2019). In 2020, Mitochon Pharmaceuticals was awarded Orphan Drug Designation by the FDA for MP101 for treating ALS (Mitochon press release, September 10, 2020). The prodrug MP201 is under development for Parkinson's disease and traumatic brain injury.

Neuroprotective Benefit: MP101/MP201 has shown neuroprotective benefits in rodent models of Alzheimer's, Parkinson's, multiple sclerosis, and brain injury. No human studies of MP101 or MP201 in neurodiseases have been completed to date.

Types of evidence:

- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No studies have tested MP101 or MP201 for the prevention of dementia or age-related cognitive decline.

Human research to suggest benefits to patients with dementia:

No studies of MP101 or MP201 have been completed in patients with dementia. In January 2024, Mitochon Pharmaceuticals announced that it was awarded approval from the European Medicines Agency (EMA) to begin enrollment for a phase I/IIa 14-day biomarker study of MP101 in Alzheimer's disease, Huntington's disease, ALS, and multiple sclerosis (Biospace article, Jan 9, 2024). The aim of the phase I/IIa study is to demonstrate safety and show changes in disease specific biomarkers.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

In normal mice, oral treatment with DNP (0.5, 1, 5, and 10 mg/kg) penetrates the blood-brain barrier and gets into the brain cortex with concentrations comparable to peripheral organs, such as the liver

and skeletal muscle, at the higher doses tested (5 and 10 mg/kg)(Geisler, 2019). Four hours after oral DNP dosing, DNP levels were highest in the liver, then skeletal muscle, then brain cortex.

In normal mice, low dose treatment of DNP (5 mg/kg/day) for 14 days significantly improved memory retention (measured by passive avoidance testing), upregulated CREB signaling (key in synaptic plasticity and learning and memory) in the cerebral cortex, and upregulated mTOR signaling, which is the central regulator of cell metabolism, proliferation, and survival (Liu et al., 2015).

Also in normal mice, treatment with low doses of DNP (1, 5, or 10 mg/kg/day, oral gavage) for 14 days significantly increased mRNA levels of BDNF in the cerebral cortex (Geisler et al., 2017).

Models of Alzheimer's disease: In a mouse model of Alzheimer's disease (APP/PS1 transgenic mice), treatment with a very low dose of DNP (0.5 mg/kg/day, oral gavage) for 4 months ameliorated hippocampal-dependent spatial learning and memory deficits (measured by the Morris water maze task)(Geisler et al., 2017). Higher doses of DNP (1 and 5 mg/kg) did not show benefits in learning and memory in this mouse model.

In a different mouse model of Alzheimer's disease induced by intrahippocampal injection of Aβ42, co-injection of DNP reduced amyloid plaque deposition by 86 ± 17%, compared to Aβ42 injection alone (De Felice et al., 2001). In rat hippocampal neuronal cultures, administration of Aβ42 caused significant cell death (survival of 25% of neurons, compared to 86% in control cultures). Co-incubation with DNP almost completely blocked the Aβ42-induced neuronal death (survival of 74% of neurons).

Models of Parkinson's disease: Rotenone and 6-OHDA are toxic agents that cause selective degeneration of dopaminergic neurons and Parkinson's disease-like motor impairment in humans and animal models. The toxins inhibit mitochondrial complex I. In a mouse model of Parkinson's disease (induced by 6-OHDA), treatment with low doses of MP201 (8 mg/kg) protected against motor dysfunction (measured by rotarod test fall latency and grip strength) and neuron loss in substantia nigra (measured by TH-positive neurons) (Kishimoto et al., 2020). Disease induction with 6-OHDA resulted in a ~70% reduction in dopaminergic inputs to the striatum in placebo-treated mice, but DNP and MP201 treatment preserved dopaminergic inputs, with reductions of only 45% and 15% of dopaminergic input, respectively. The protective effects of MP201 were greater than those of DNP (0.5 mg/kg).

Also in this mouse model of Parkinson's disease, microglial and astrocytic reactivities were increased in the substantia nigra (measured by Iba1 and GFAP immunoreactivity), but DNP and MP201 suppressed

microglial and astrocytic reactivities, with MP201 being more effective in suppressing the reactivities (Kishimoto et al., 2020).

Models of multiple sclerosis: Multiple sclerosis is an autoimmune disease in which myelin-reactive autoantibody and lymphocytes migrate out of lymph nodes into the circulation, cross the blood–brain barrier, and target brain myelin antigens, causing inflammation, demyelination, axonal injury, astrogliosis, and neurodegeneration. In a mouse model of multiple sclerosis (induced by EAE), treatment with MP101 (5 mg/kg, oral gavage; provided by Mitochon Pharmaceuticals) or MP201 (8, 16, or 80 mg/kg; provided by Mitochon Pharmaceuticals) on days 7 to 21 post-immunization, significantly delayed onset of symptoms, reduced the severity of symptoms, suppressed progression of paralysis, sped up the recovery, reduced demyelination, reduced axonal degeneration, and limited the infiltration of inflammatory cells (Bando and Geisler, 2019). MP201 exhibited dose-dependent effects and the effects were superior to those of MP101.

MP101/MP201 treatment in EAE mice also induced a significant and sustained increase in the level of BDNF in the spinal cord, suppressed the expression of inflammatory cytokines including IL-1β, TNF-α, and iNOS, and improved the health of axonal mitochondria (longer mitochondrial length and fewer abnormally shaped mitochondria)(Bando and Geisler, 2019). Although mitochondrial uncouplers are associated with weight loss at high doses, MP101 and MP201 preserved body weight at all doses in comparison to the wasting observed in the placebo group.

Cuprizone is a chelating agent of copper and when ingested, it lowers the essential dietary copper, preventing the formation of myelin sheaths by oligodendrocytes. Cuprizone slowly induces demyelination by oligodendrocyte cell death in the mouse brain. In a mouse model of multiple sclerosis induced by cuprizone, treatment with MP101 or MP201 significantly preserved myelination of axons in the corpus callosum by reducing glial activation (Bando and Geisler, 2019).

Optic neuritis, characterized by optic nerve inflammation, demyelination, and axonal loss, is often associated with multiple sclerosis. Mitochondrial dysfunction and inflammation appear to play roles in the neurodegeneration of optic neuritis. In a mouse model of optic neuritis (induced by EAE), treatment with MP201 (16 or 80 mg/kg, once daily, provided by Mitochon Pharmaceuticals) from day 15 post-immunization and onward, attenuated visual dysfunction (measured by optokinetic responses), preserved retinal ganglion cells, reduced retinal ganglion cell axonal loss in the optic nerve (measured by neurofilament staining), and reduced demyelination in the optic nerves (Khan et al., 2017). In EAE mice, those that received the 80 mg/kg dose of MP201 showed vision loss at week 3 that reversed and

improved significantly at later time points. EAE mice that received the 16 mg/kg dose of MP201 showed a significant improvement in optokinetic response scores at all time points compared to vehicle-treated mice. Optic nerves of EAE mice treated with 16 mg/kg and 80 mg/kg doses of MP201 showed significant inflammatory cell infiltration similar to that seen in vehicle-treated EAE mice, with no statistical differences across groups.

***Models of Huntington's disease*:** In a mouse model of Huntington's disease (induced by mutant huntingtin), DNP treatment (1 mg/kg/day) for 17 weeks significantly improved motor function (measured by rotarod test) and preserved medium spiny neurons (measured by levels of DARPP32) and synapses (measured by levels of PSD95) in the striatum, while reducing oxidative stress (measured by F2-isoprostanes) (Wu et al., 2017).

Models of brain injury: Traumatic brain injury can result in long-term cognitive impairment. In a mouse model of traumatic brain injury (cortical impact), MP201 treatment (80 mg/kg, oral gavage; provided by Mitochon Pharmaceuticals) started 2 hours post-injury and continued daily for 2 weeks significantly improved cognitive function (measured by novel object recognition test), increased cortical sparing, prevented the loss of hippocampal CA3 neurons, improved mitochondrial bioenergetics (increased complex I- and complex II-mediated respiration), and reduced oxidative markers (4-hydroxyneneal and protein carbonyls), (Hubbard et al., 2018).

In a rat model of military-relevant repeated mild blast traumatic brain injury (induced by repeated blast of 11 psi peak overpressure), treatment with MP201 (80 mg/kg, orally) rescued impairments in synaptic mitochondrial respiration in the prefrontal cortex and amygdala/entorhinal/piriform cortex (Hubbard et al., 2023). Synaptic mitochondrial respiration in the hippocampus was not rescued by MP201 treatment. Although MP201 treatment alleviated the elevated mitochondrial oxidative damage in glia, effects depended on the timing and dosage of MP201 treatment.

Models of cerebral ischemia: In a rat model of cerebral stroke (induced by the occlusion of the carotid artery and middle cerebral artery for 2 hours), treatment with DNP (5 mg/kg, i.p.) 1 hour after the reperfusion reduced infarct volume by approximately 40% (Korde et al., 2005). The mechanism of neuroprotection involved an early decrease in the formation of mitochondrial reactive oxygen species and calcium uptake, leading to improved mitochondrial function (measured by respiratory control ratio).

APOE4 interactions: Unknown.

Aging and related health concerns: DNP was used as a weight loss agent until it was banned due to toxicity. In rodents, doses of DNP that are up to 100-fold lower increased longevity, and improved metabolic indices, hearing loss, and nerve injury.

Types of evidence:

- Clinical trials from the 1930s testing high doses of DNP for weight loss
- Several laboratory studies

Lifespan: INCREASED LIFESPAN IN MICE

In mice, DNP in drinking water (1 mg/L) started at 18 weeks of age significantly enhanced lifespan, mildly reduced weight gain, and decreased oxidative stress in the brain (measured by reactive oxygen species, DNA damage, and protein oxidation)(Caldeira da Silva et al., 2008). Median longevity in the control group was 722 days, while the median longevity in the DNP-treated group was 771 days. The mean lifespan was 718.8 days in the control and 769.7 days in the DNP-treated group. DNP treatment did not alter food or water intake or body temperature. The dose of DNP used in this study (100 µg/day) is equivalent to a human dose of 0.45 mg/day. In contrast, the dose used in the 1930s for weight loss that was associated with toxicity was ~300 mg/day.

Metabolic diseases: USED AS WEIGHT LOSS AGENT UNTIL BANNED

Historically, DNP was used in the 1930s as a weight loss agent in obese patients (reviewed in Geisler, 2019). The first clinical study with DNP was published in 1933, and within a year, well over 100,000 people were treated with DNP in the US alone (Tainter et al., 1934). Typically, a DNP dose of 100 mg/day resulted in a weight loss of ~1 lb per week, and doses were increased to ~300 mg/day (100 mg, 3 times daily) to achieve a weight loss of 3 lbs per week (Geisler, 2019). High doses were associated with toxicities, such as rashes, cataracts, and even death. In 1938, the Food, Drug, and Cosmetic Act was established, and DNP became illegal to purchase. However, DNP is still used today on the black market, for example, by weightlifters who are seeking to remove the fat between muscle groups to increase definition.

In mice, a very low dose of DNP in drinking water (1 mg/L) started at 18 weeks of age significantly improved serum glucose, triglyceride, and insulin levels (Caldeira da Silva et al., 2008).

Peripheral neuropathy: POTENTIAL BENEFIT IN A MOUSE MODEL
In a mouse model of sciatic nerve injury (induced by a crush injury), treatment with a very low dose of DNP (0.06 mg/kg, i.p.) immediately after the trauma, and an additional dose 24 hours later, significantly reduced both the edema and axonal degeneration (da Costa et al., 2010). When mice with sciatic nerve injury were treated with 4 doses of DNP (0.06 mg/kg, i.p., every 12 hours post-trauma), nerve damage was almost absent and difficult to distinguish from the sham control group. At 6 weeks post-injury, mice treated with DNP showed partial limb function (paw toe extensions), while the placebo-treated mice showed no recovery.

Hearing loss: POTENTIAL BENEFIT IN A RAT MODEL
In a rat model of hearing loss (exposed to 105 dB noise for 8 hours), MP201 treatment (80 mg/kg/day, orally) for 5 days, and to a lesser extent, MP101 treatment (5 mg/kg/day, orally) for 5 days, preserved auditory function assessed by the compound action potential (Geisler, 2019). The compound action potential amplitudes were larger (better) in the noise-exposed groups that received MP201 versus the noise alone group, and the differences were statistically significant at 16, 20 and 24 kHz ($p<0.0001$), but not at 12 kHz.

Safety: High doses of DNP were used for weight loss, which caused dangerous adverse events including death. Doses of MP101 that show protective benefits in mouse models are ~10-100 times lower. No clinical trials of MP101/201 have been completed to date.

Types of evidence:

- Numerous review articles
- Numerous laboratory studies

No clinical trials of MP101 or MP201 have been completed in humans. Safety of very low-dose DNP (=MP101) or its prodrug MP201 has not been established yet.

In the 1930s, DNP at high doses was used as a weight loss medication, but caused many dangerous adverse events, including death, and its use was banned as a prescription drug (reviewed in Geisler, 2019). Doses commonly used in the 1930s for weight loss was ~100 mg, three times daily (300 mg per day). Toxicities included rashes and cataracts. Rashes resolved with discontinuation of DNP, but cataracts required surgery to replace the lens. The number of people who were taking DNP (at doses recommended for weight loss) who were affected by cataracts exceeded 164 (Horner, 1941). Of these

people, 98% were women who were on average 45 years old. Overdose of DNP produces a combination of symptoms including hyperthermia, tachycardia, diaphoresis, tachypnea, and possibly death. The lowest published lethal human oral dose of DNP was 4.3 mg/kg (Grundlingh et al., 2011). To date, there have been 62 published deaths in the literature that are attributed to DNP. In documented fatalities relating to DNP exposure, doses of DNP ranged from 2.8 g to 5.4 g, as a single dose or used across days/weeks.

Doses of MP101 that have shown neuroprotective benefits in mouse models have been ~10-100 times lower than the DNP dose used in the 1930s for weight loss. For example, in a mouse model of Alzheimer's disease, treatment with DNP at a dose of 0.5 mg/kg/day ameliorated learning and memory deficits (Geisler et al., 2017). The human equivalent dose is 0.041 mg/kg/day, which, for a person weighing 80 kg (~176 lb) would be 3.25 mg per day. Similarly, MP201, the prodrug of DNP, has been tested at doses that generate ~10-50 times lower levels of DNP than the DNP dose used for weight loss (Khan et al., 2017).

Mitochon sponsored work to compare the pharmacokinetics of MP101 and MP201, and found that the absorption and conversion of MP201 to MP101 is slowed, and the Cmax of MP201 was up to 20-fold lower when comparing 5 mg/kg of MP101 to the equivalent of MP201 (after adjusting for molecular mass)(reviewed in Geisler, 2019). Compared to MP101, MP201 also has an extended area under the curve by up to 10-fold and residency time of up to 3-fold.

Drug interactions: Drug interactions with MP101 or MP201 have not been documented.

Sources and dosing:

MP101 is under clinical development by Mitochon Pharmaceuticals for the treatment of amyotrophic lateral sclerosis (ALS), Huntington's disease, and multiple sclerosis (Mitochon pipeline). The prodrug MP201 is under development for Parkinson's disease and traumatic brain injury. Dosing has not been established for MP101 or MP201 in humans. Doses of MP101/MP201 that have shown neuroprotective benefits in mouse models have been significantly lower than the DNP dose used in the 1930s for weight loss.

Research underway:

In January 2024, Mitochon Pharmaceuticals announced that it was awarded approval from the European Medicines Agency (EMA) to begin enrollment for a phase I/IIa 14-day biomarker study of MP101 in ALS, multiple sclerosis, Huntington's disease, and Alzheimer's disease (Biospace article, Jan 9, 2024). The aim of the phase I/IIa study is to demonstrate safety in the target patient populations and show changes in disease specific biomarkers. The National Institute of Neurological Disorders and Stroke (NINDS) is funding a program testing MP201 in preclinical models of traumatic brain injury (R01 NS112693).

Search terms:
Pubmed, Google: MP101, MP201, 2,4-Dinitrophenol

Websites visited for MP101, MP201:

- Clinicaltrials.gov (0)
- NIH RePORTER
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca