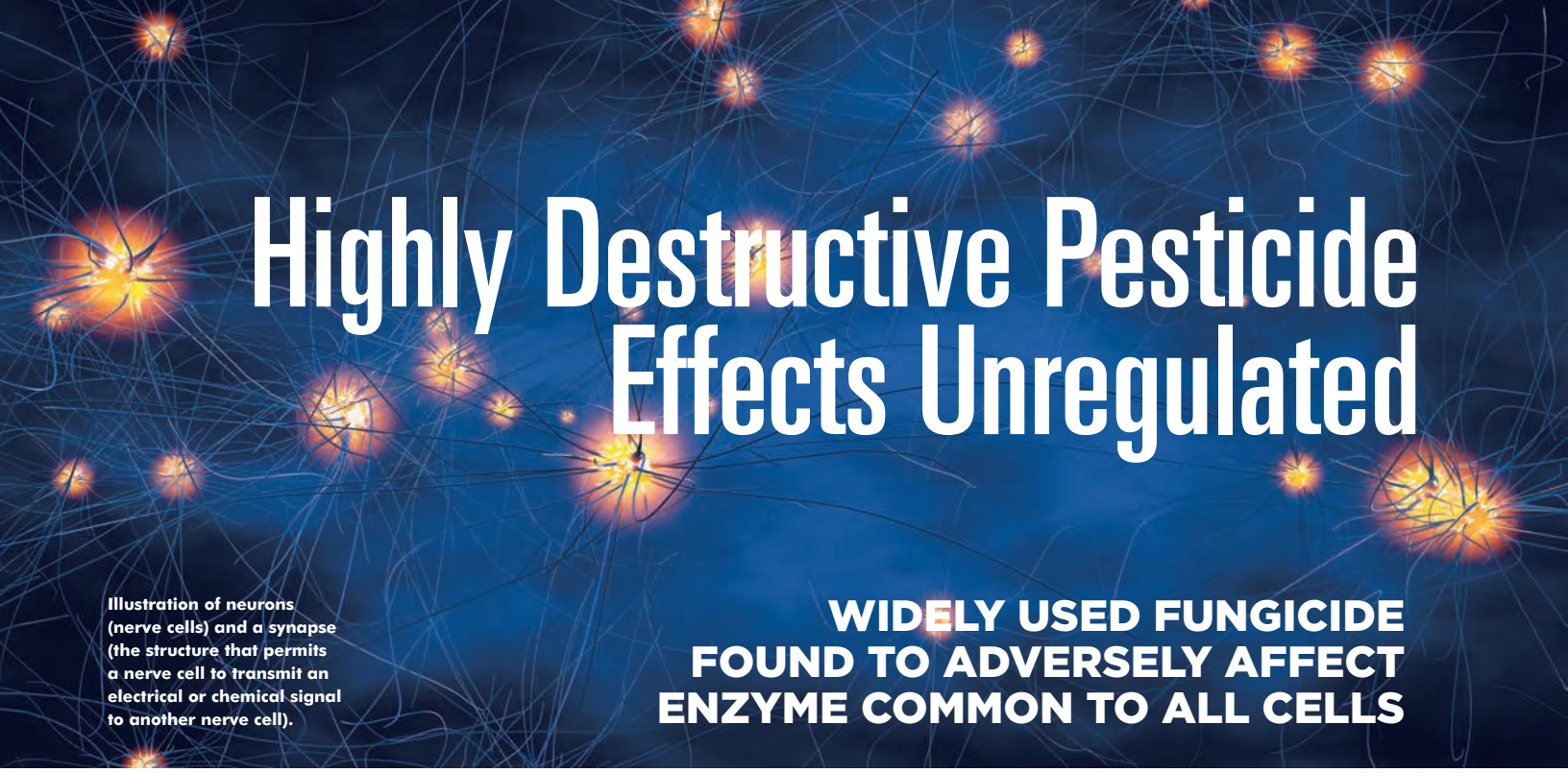


Highly Destructive Pesticide Effects Unregulated

Illustration of neurons (nerve cells) and a synapse (the structure that permits a nerve cell to transmit an electrical or chemical signal to another nerve cell).

WIDELY USED FUNGICIDE FOUND TO ADVERSELY AFFECT ENZYME COMMON TO ALL CELLS

DEBRA SIMES

EDITOR'S NOTE. *To our non-scientist readers, we urge you to wade through this article and share it with those making decisions on pesticide use and food choices. The take-home message from the research discussed here is that the complexity of pesticide effects on cells and enzymes in the body, and organisms generally, are not understood to the extent that they should be. And yet, people in positions of authority push for pesticide use, sometimes because they simply defer to regulators, believe that because a pesticide is on the market it must be safe, or have been trained to use the chemicals. Sometimes, without knowing the science, or lack of science, they feel that the pest control needs are more important than any potential harm, known or unknown, of the pesticide use. In this piece, the unknowns associated with the chemical effect that is the subject of this article turns out to have dramatic and frightening effects. —Jay Feldman*

This is a story about a chemical pesticide, a fungicide, in wide use for which the mode of action, i.e., the ability to cause harm, has not been fully understood. It is not a story unique to this pesticide. Rather, it is an important reality to consider when deciding to use a pesticide or allowing a pesticide to be used. The question is whether the chemical could be broadly problematic beyond the target organisms, in this case fungi?

In its coverage of a study published in March, 2019, the American Association for the Advancement of Science publication, EurekAlert, reported that, "The ability of [the fungicide] fludioxonil to act on a sugar-metabolizing enzyme common to all cells, and to produce the damaging compound methylglyoxal, may mean that the pesticide has more potential to harm non-fungal cells than previously thought. Although fludioxonil has been deemed safe for use, the authors…suggest that the effects of this widely used pesticide has upon animals be re-examined."

The research study, "Phenylpyrrole fungicides act on triosephosphate isomerase to induce methylglyoxal stress and alter hybrid histidine kinase activity," published in *Scientific Reports* and led by T. Tristan Brandhorst, PhD (in the lab of Bruce Klein, PhD, University of Wisconsin–Madison and UW School of Medicine and Public Health), sheds light on that mechanism and raises the alarm about implications of the discovery.

THE USE AND HISTORY OF A FUNGICIDE

Among the myriad pesticides used in agriculture is fludioxonil, a phenylpyrrole fungicide, which was developed to treat seeds during storage. However, it has come to be used commonly on grains, vegetables, fruits, and ornamental plants during cultivation, and, making it even more widespread (more on this below), to treat produce after it has been harvested to extend "shelf life." Though fludioxonil is effective in killing fungi, the mode, or mechanism, of action for this pesticide was previously not well understood.

Fludioxonil was introduced in 1993–1994 by Ciba-Geigy (now Syngenta), and pesticides that include the compound are now marketed under various brand names, including Cannonball, Switch, Medallion, Helix, Celest, Apron, Agri Star Fludioxonil, Dyna-shield Fludioxonil, Maxim, Scholar, Spirato, and others. Syngenta promotes it for use on "targeted fungi, such as snow mold, seedborne and soilborne *Fusarium*, [and] seedling blights or bunts." Its use has increased in the 25 years since its introduction, and particularly in the last few, ratcheting up concern about its features and impacts.

THE MECHANISM CAUSES CELL DEATH

In a previous investigation, Drs. Brandhorst and Klein pointed to the uncertainty about how fludioxonil actually causes fungi

cell death, asserting that this uncertainty merits a reevaluation by the U.S. Environmental Protection Agency (EPA) of its potential impacts on human health, noting reports of the fungicide's ability to disrupt hepatic (liver), endocrine, and neurological systems. Prior to this current study, it was believed that fludioxonil targets hybrid histidine kinase (HHK), a protein in fungal cells. Regarding the mechanism of action, Syngenta has theorized that fludioxonil binds to HHK, activating a biochemical process that causes fungal cells to kill themselves. In 2016, Dr. Klein's lab team found that, although fludioxonil needs HHK in order to kill fungi, the pesticide and protein do not directly interact.

The scientists in Dr. Klein's lab turned to the hypothesis that oxidative stress—a common effect of pesticides on their targets—might be the linchpin. (Oxidative stress is an imbalance between cells that are oxygen-producing free radicals and antioxidant defenses in the body.) Yet, the team found that, when they exposed fungi to various kinds of oxidative stress, cells remained healthy. Then, the researchers discovered that fludioxonil inhibits an enzyme related to cellular sugar metabolism, causing (via a spike in methylglyoxal release) activation of the deadly HHK cascade.

DAMAGING ALL CELLS

Dr. Brandhorst notes, "The take home lesson is that fludioxonil is multifactorial. It is not compromising cells by one solitary mechanism. It has potential to damage cells in a variety of ways." He references a 2007 investigation that demonstrated that, in fungi, disruption of glutathione homeostasis (which manages oxidative stress) synergistically enhances the toxicity of fludioxonil, suggesting that an oxidative stress response pathway may overshadow osmoregulation functions (maintenance of constant osmotic pressure in the fluids of an organism by the control of water and salt concentrations). Glutathione is primarily an intracellular antioxidant, which protects cells against the effects of free radicals—which can include damage to DNA. Fludioxonil has been shown to have DNA damaging impacts on human liver cells, and Dr. Brandhorst suspects that glutathione depletion (a signaling event that regulates the activation of cell death pathways) may ultimately be identified as a factor in fludioxonil-related hepatic (liver) damage. The enzyme-suppressing action of fludioxonil on an enzyme common to all cells is at the heart of the alarm this research is raising, but it is not the only reason the fungicide needs to be reevaluated.

PERSISTENCE, TOXICITY, AND ESCALATING TOXICITY

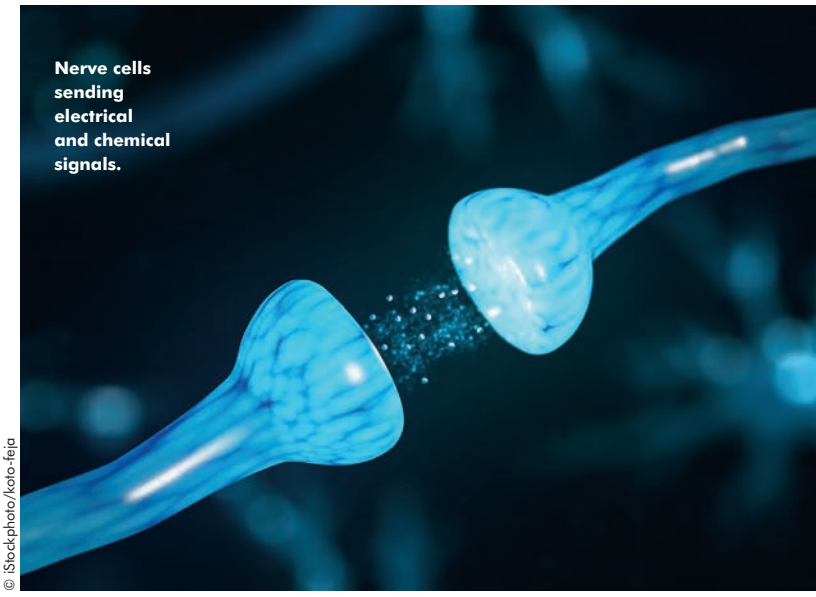
Fludioxonil persists in soil—near the surface for weeks, and for years if it ends up deeper in the soil, where sunlight cannot speed its degradation; it is also a "super toxin" for earthworms. The fungicide's extensive post-harvest use on food crops is of particular concern because it eliminates the chance for wind, rain, and ultraviolet-visible (UV-vis) light to break down the compound, and once applied, the waxy fungicide is not easily removed by rinsing. Further, UV-vis treatment of produce (which is sometimes done to reduce pathogens on fresh fruits and vegetables) actually significantly increases the toxicity of fludioxonil.

The enzyme-suppressing action of fludioxonil on an enzyme common to all cells is at the heart of the alarm this research is raising, but it is not the only reason the fungicide needs to be reevaluated.

The fungicide also is an EPA Category I toxic substance—"highly toxic and severely irritating"—to aquatic plants, bacteria, insects, fish, and aquatic invertebrates, generating concern about its use near water bodies or shorelines. Beyond all that, the lead author indicates that "there is also reason to believe that breakdown products [new chemicals formed in the environment] of this pesticide may be 100 times more toxic than fludioxonil itself."

SYNERGY NOT EVALUATED

In addition, the issue of synergistic action among multiple pesticide compounds, or active and adjuvant ingredients in a pesticide, is woefully under-addressed by regulators. Synergistic action was explained simply and long ago by Beyond Pesticides in its journal *Pesticides and You*: "The concept of interaction is fundamental to understanding the processes by which chemical mixtures act. If the effect is simply additive, the sum of the effects is the same as if we were exposed to each chemical individually. Synergy occurs when the effect



Nerve cells sending electrical and chemical signals.

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of a mixture of chemicals is greater than the sum of the individual effects."

REGULATORY FAILURES

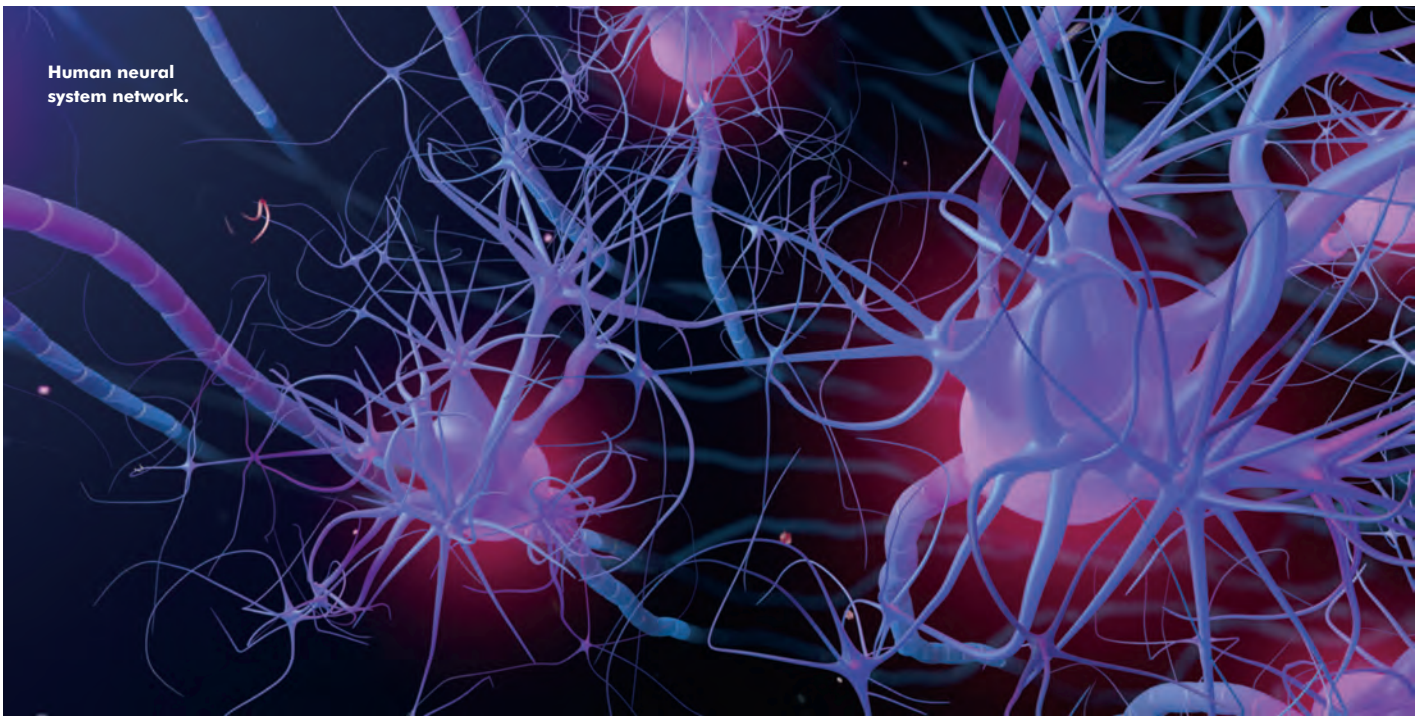
The federal bodies in the U.S. that are supposed to ensure the safety of both chemicals used in the environment, and those used on food crops and products—EPA, and the Food and Drug Administration (FDA), respectively—fail to do so. Another passage from the *Pesticides and You* article offers background: "In 1996, EPA was required for the first time to consider cumulative pesticide exposures in limited circumstances under the *Food Quality Protection Act* (FQPA). [That Act], which amends the *Federal Insecticide, Fungicide and Rodenticide Act* and the *Federal Food, Drug, and Cosmetic Act*, recognizes that real-world pesticide exposures do not occur as single discrete exposures to a specific pesticide, but rather in combination [with] several pesticides at once.... To address the issue of multiple pesticide exposures, FQPA directs EPA to consider combinations of pesticides that have a common mechanism of toxicity when setting tolerances" ["acceptable" levels of pesticide residue in agricultural products]. Because this statutory mandate is a narrow one, confined to compounds that have a "common mechanism of toxicity," many chemicals are never evaluated by EPA for their synergistic potential.

Thus, EPA continues not to evaluate comprehensively for synergistic effects, which can be more toxic than exposure to a single compound. In 2016, the Center for Biological Diversity wrote a report on this: *Toxic Concoctions: How the EPA Ignores the Dangers of Pesticide Cocktails*. Adding to the concern about fludioxonil's mechanism of action and the implications for all organisms, including humans, is its synergistic potential. A 2012 study by French researchers found that a mixture of fludioxonil and cyprodinil, another fungicide, yields data suggesting cytotoxic (lethal to cells) and genotoxic (damaging to DNA) effects at low concentrations, and with a significantly higher effect of the mixture than would be expected from an exposure response to the individual fungicides. This study by Dr. Brandhorst, et al. adds to the growing body of research on the interactive effects of pesticides on human health and the environment.

RESPONSE: TAKING A PRECAUTIONARY APPROACH

Beyond Pesticides advocates for a far more precautionary approach to pest management in land management and agriculture, with a transition to organic methods as the ultimate goal. In 1998, a gathering of scientists, philosophers, lawyers, and environmental activists produced this statement on the Precautionary Principle (known as the Wingspread Statement):

> When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically. In this context the proponent of an activity, rather than the public, should bear the burden of proof. The process of applying the precautionary principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action.*



Human neural system network.

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* Wingspread statement.1998. *https://www.sehn.org/sehn/wingspread-conference-on-the-precautionary-principle*.