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Health Effects of Neonicotinoids on an Agricultural Community

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Master's Program of Public Health: Environmental and Occupational Health

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Background and Purpose:

Neonicotinoid insecticides, popularly known for their destruction of wild and domestic bee populations, are seen as "safe" pesticides to use in the agricultural industry. However, within the last 15 years, scientists have found many potential human health risks associated with acute and chronic exposures of neonicotinoids. Although, there are training and personal protective equipment for agricultural workers to help lower their exposure to these chemicals, neonicotinoids are still finding their way into the human body of people not directly working with the compounds (Laubscher, B., 2019, Ospina, M. et al., 2019, Harada, K. H., 2016).

Mead, Nebraska, a small United States agricultural community consisting of approximately 600 people, was accidentally exposed to high amounts of neonicotinoids when AltEn, an ethanol plant that used neonicotinoid coated corn and soybean seeds for biofuel, was discovered not disposing of the toxic byproduct safely. Coincidentally, there was also a leak in their wastewater system that also contaminated the nearby areas. Although neonicotinoids are not considered highly dangerous with low dose exposures, large dose exposures can cause symptoms similarly to nicotine poisonings, such as difficulty breathing, vomiting, nausea, hypertension, tachycardia, and lethargy (Imamura, T. et al., 2010). Neonicotinoid poisoning can be fatal as it impacts multiple organ systems, and there is no standard practice of care to treat such poisonings as symptoms amongst cases are variable (Lin, P. et al., 2013).

Current research suggests that neonicotinoids are linked to several toxicities such neurotoxicity, reproductive toxicity, renal toxicity, genotoxicity, and cardiotoxicity. The main goal of this review was to compile current research from the last 10-15 years on each of the neonicotinoids and display the human health effects that each of the compounds may cause. The minor goal was to analyze data collected from an environmental health risk perception survey

given to the Mead community to see any association between what people reported experiencing from the AltEn event and different neonicotinoid health effects that can occur from acute and chronic exposure.

Neonicotinoid Overview:

Neonicotinoids are a type of insecticide developed in the 1990's with Imidacloprid being the first neonicotinoid on the market in 1994. Neonicotinoids were seen as a substitute for the highly toxic organophosphates that had been utilized since 1932 (Frumpkin, 2016). Many of the neonicotinoids are derivatives of the compound Nithiazine which targets the nicotinic pathway by mimicking nicotine's chemical properties (Pang, S. et al., 2020). Due to the modifications to make a more photostable compound, neonicotinoids are very water soluble and have the potential to linger in ground water once applied to crops or once coated seeds are planted. This leads to a chemical buildup of the compounds in the soil and underground water channels or systems when the crops are treated multiple times during the year (Bai, A. et al., 2021, Kurwadkar, S. et al., 2016, and Ospina, M. et al., 2019).

The chemicals repel insects by biologically affecting their nicotinic acetylcholine receptors (nAChRs), which are found in the neuromuscular and reproductive system (Phogat, A. et al., 2022). Neonicotinoids bind to and activate nAChR but are unable to be inactivated by the acetylcholine esterase (AChE) due to the metabolites structure forcing the central nervous system to be overstimulated (Buszewski, B. et al., 2019). A visual representation is seen in Fig 1. Overstimulation

Figure 1. a.) Displays how nAChR, represented in yellow, typically reacts to acetylcholine, represented in orange, and how AChE, represented in red, is able to bind to acetylcholine enabling inactivation of the nAChR and acetylcholine degradation. B.) Displays how nAChR, represented in yellow, reacts with neonicotinoids, labeled in figure, and how AChE is unable to bind to it leaving nAChR active.

causes neurological dysfunctions such as paralysis, lethargy and limb spasms, starvation, and death (Cabirol, A. et al., 2019). Because neonicotinoid metabolites affect the nicotine receptors in insects, they target the same central nervous system pathway along with the parasympathetic pathways in humans (Klaassen, Curtis D. et al., 2019). Common symptoms associated with neonicotinoid poisoning are fatigue, headaches, nausea, respiratory problems, vomiting, hypertension, and tachycardia (Lin, P et al., 2013). There have been claims that the effects of neonicotinoids may cause neurological damage in a similar fashion as Alzheimer's disease due to the nAChRs activation because of the receptors involvement in neuron development and life cycle (Buszewski, B. et al., 2019). Others have shown that there is a potential link to neurological dysfunction (Nakayama, A., 2019, Hirano, T., 2019, Kagawa, N., 2018, Sheets, L., 2016, and Taria, K., 2021).

For this review, Acetamiprid, Dinotefuran, Imidacloprid, Thiacloprid, Thiamethoxam, and Clothianidin were examined to compile the current health hazards found by other researchers. From that collected knowledge, this review will examine the survey data from Mead and see if there is a possible connection.

Acetamiprid:

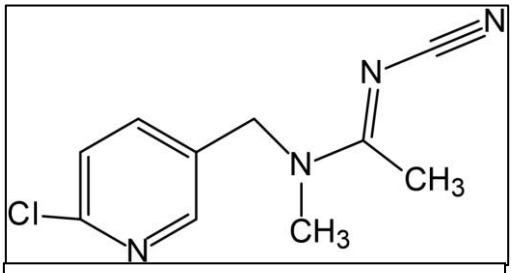


Figure 2. The basic chemical structure of Acetamiprid.

Acetamiprid (Figure 2), also known as Assail, Pristine, or Chipco, is a neonicotinoid that is applied via broadcast or direct foliar spray to leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton, and ornamental plants and flowers mainly sold as a powder or an aqueous solution (Phogat, A. et al., 2022). It has a half-life of 34 days in water that has a pH 7 at 25°C and its half-life in soil, estimated based on photodegradation and aerobic conditions, is 8-9 days (EPA, n.d.). Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid. While farmers have personal protective equipment while applying these chemicals to the crops, the consumers are exposed through ingestion. Many recent research studies have discovered that many of the compounds' metabolites reside heavily in the blood, stomach, and liver tissue rather than kidneys after ingestion and can be measured in urine excretion (Harada,K.H., 2016, Phogat, A. et al., 2022). Because of its chemical structure Acetamiprid can go through several chemical reactions once it enters the body such as demethylation, deacetylation, and hydrolysis of the cyano-imine linkage (Phogat, A. et al., 2022).

A deeper analysis of the metabolism of acetamiprid suggests multiple different systemic impact points. When looking at basal or apical pathways, if the sodium transport system in cells is inhibited, the uptake of acetamiprid is lower and the toxic effects are lessened. If ATP is heavily depleted and trypsin is added in, the cells have a higher uptake of acetamiprid (Phogat, A. et al., 2022). When looking at the quantity of metabolites in the body, acetamiprid has a direct

relationship to the toxic symptoms experienced. Common acetamiprid poisoning symptoms such as memory dysfunction, respiratory failure, vomiting, nausea, hypotension, convulsions, muscle weakness, and hyperthermia have all been reported with variability of severity based on the dose of acetamiprid. In one study, patients with the highest dose (6 ppb) of the metabolite N-desmethyl-acetamprid found in their urine had worsened symptoms ranging from memory loss, finger tremors, fatigue, palpitations, coughing, and headaches compared to the patients who had 2 ppb of the metabolite and were asymptomatic (Phogat, A. et al., 2022).

Not only does the metabolism of acetamiprid have direct physical effects, but some studies have also found chronic exposure has reproductive and neurological effects as well. One study discovered that there is a dose-response linkage between high doses of the compound and lower fertility in male rats. This can be seen in a lower overall sperm count in highly exposed groups compared to lower dosed groups (Arican, E., 2020). Additionally, in the groups that have high dose exposure (35 mg/kg per day for 90 days), the sperm physicality changes from a rounded head to a more flattened head, which potentially impacts the motility of the sperm (Arican, E., 2020). It also has a negative effect on placental and fetal growth and may lead to the development of dwarfism, eye abnormalities, hemorrhage, underdevelopment in the lungs and heart hypertrophy, and structural anomalies of the skeletal system, i.e., ossification and shortened ribs (Phogat, A. et al., 2022). In human studies, reduced sperm counts, and stillbirths have been associated with acetamiprid doses (Phogat, A. et al., 2022).

Additionally, acetamiprid also has been shown to have neurological effects on offspring of rodents that have been exposed to the compound during pregnancy and after birth. It was reported that hypoplasia increased due to decreased neural stem cell cycle arrest in the hippocampal dentate gyrus (Nakayama, A., 2019). This indicates that with prenatal exposure to

acetamiprid there is an association of decreased structural plasticity in the hippocampus. Furthermore, M1 microglia signaling was increased, showing an imbalance between M1 microglia, the inflammatory mediators, and M2 microglia, the anti-inflammatory mediators, in the brain, which affects the developing neocortex of the offspring from the adult mice that ingested acetamiprid during gestation (Nakayama, A., 2019). Other evidence using pc12 cells has shown that there is neurotoxicity by showing a dose response of DNA fragmentation. The longer the cells were exposed to the compound, the more DNA damage, and the more reactive oxygen species (ROS) occurred, but because the apoptotic process that acetamiprid triggers is caspase dependent, researchers believe that ROS was impacting the DNA fragmentation process (Annabi, E., 2019).

Dinotefuran:

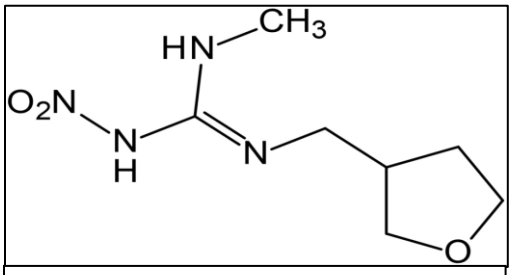


Figure 3. The chemical structure of Dinotefuran.

Dinotefuran (Figure 3), more commonly known as K9-Advantage flea and tick repellent, is a neonicotinoid that not only is used in veterinary medicine, but also used on leafy vegetables, in residential and commercial buildings, outdoor uses for professional turf management, turf farms, professional ornamental production, golf courses, residential indoor, lawn and garden use, and foliar application on crops (EPA, n.d.). It has a half-life in light and aqueous conditions of 2 days, whereas in the soil and anaerobic conditions its half-life is 50-100 days (EPA n.d.). Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid. No carcinogenesis risk is

associated with Dinotefuran, and few health risks are associated with it due to the chemical structure highly selecting for the insect nAChRs.

Signs of acute toxicity in rats show decreased motor skills after being exposed to Dinotefuran along with other neurological and reproductive toxicity (EPA n.d.). Studies show a mental health connection to Dinotefuran, such as depression and anxiety (Takada, T., 2020). One study explored chronic stress and exposure to Dinotefuran which showed that mice exposed to Dinotefuran increased the corticosterone levels, altered the mouse's behavior, and lowered neurotransmitter levels at the NOEL dose (Takada, T., 2020). Reproductive toxicity is also associated with Dinotefuran effects like abnormal sperm shape and motility found in male rodents, dogs, and birds, along with larger ovaries and altered cycles in females (Takada, T., 2020 and EPA n.d.).

Imidacloprid:

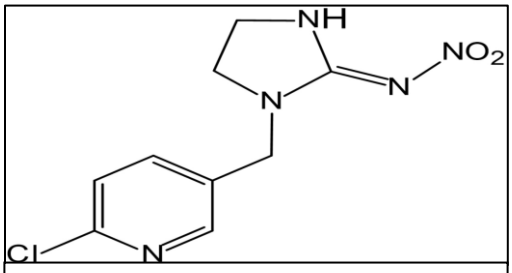


Figure 4. The chemical structure of Imidacloprid.

Imidacloprid (Figure 4), also known as Gaucho, can be applied directly to the plant or animal in need of pest repellent or can be coated directly onto crop seeds before planting. It has a half-life when in water and exposed to light of an hour while when in anaerobic soil exposed to light its half-life is 40-126 days (EPA n.d.). The most common route of exposure is by ingestion. Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid.

Imidacloprid has many health risks associated with high dose, chronic, and acute exposures. Involved with all types of exposure is the formation of Nitric Oxide (NO). When exposed to Imidacloprid, the NO product gets elevated in the liver, brain, and plasma (Duzguner,

V., 2010). This increase of NO causes a proinflammatory cytokine response and oxidative stress and subsequently causes lipid peroxidation (Duzguner, V., 2012). Lipid peroxidation abundance can trigger apoptosis causing an increase of cell death in the liver and brain. Because of oxidative stress there is a decrease in glutathione, a ROS homeostasis scavenger, which, in the brain, can cause neurodegeneration like Parkinson's and Alzheimer's diseases (Duzguner, V., 2010, Duzguner, V., 2012, and Aoyama, K., 2021).

Not only does this compound cause NO production, but reproductive toxicity is also associated with chronic and/or high exposures to the Imidacloprid. This compound impacts sperm count, physiology, and motility, but also induces fatty acids and lipid peroxidation in the testes (Bal, R., 2012). This impairs fertility by inducing high rates of germ cell apoptosis and low levels of testosterone (Najafi, G., 2008). Because of the high rates of germ cell apoptosis, oligonucleotide fragmentation can be separated out to show that with higher doses of Imidacloprid in male rats (8mg/kg per day for 3 months), more DNA fragmentation was found in the testes and sperm. (Han, W., 2018). In female rats, there is a noticeable change in ovarian morphology coupled with hormone dysregulation and induced oxidative stress when subjected to high chronic doses (20mg/kg per day for 90 days) of Imidacloprid (Kapoor, U. 2011). Additionally, when looking at neurotoxicity *in utero* of female rats, a single large dose (337mg/kg in corn oil) of Imidacloprid during gestion resulted in neurobehavioral deficits and a high amount glial fibrillary acidic protein (GFAP) in the brain which could potentially cause long term health effects in the offspring as they age (Abou-Donia, M.B., 2008). A human study found that mothers exposed to higher amounts of Imidacloprid had smaller fetal head circumferences, a measurement that is believed to be related to cognitive and neurological development for children (Villar, J., 2021).

Thiacloprid:

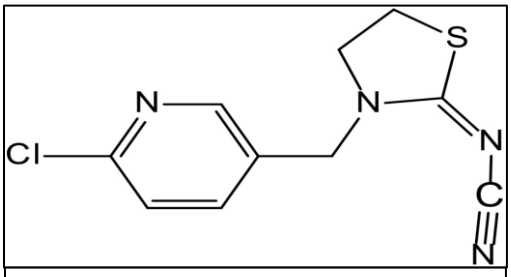


Figure 5. The chemical structure of Thiacloprid.

Thiacloprid (Figure 5), also known as Calypso, is a neonicotinoid applied to cotton and pome fruit crops by foliar spraying. It has a half-life of over 1 year in anaerobic aqueous conditions or 10-63 days if in aerobic aqueous conditions and in soil it's half-life ranges from 5 to 27 days (Pang, S. et al., 2020 and EPA n.d.). Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid. Thiacloprid breaks down similarly to acetamiprid, meaning that the compound can go through demethylation, deacetylation, and hydrolysis of the cyano-imine linkage (Pang, S. et al., 2020). There are many health and toxic hazards associated with this compound such as genotoxicity, reproductive and neurodevelopmental toxicity, and possible carcinogenesis.

An increased risk of cytokine proinflammation occurs in the liver and brain from an increase in ROS formation after exposure to Thiacloprid (Galdíková, M, 2019). An overexpression of proinflammatory cytokine responses can lead to lipid peroxidation, which causes the cells to undergo apoptosis (Duzguner, V., 2012). However, Thiacloprid does not only induce apoptosis, but the compound also plays an effect on the cell cycle (Schwarzbacherová, V., 2019). Several studies have discovered that Thiacloprid slows down the cell cycle and after 24h exposure causes the cells to stay mostly in G0 or G1 phase, meaning that cells are no longer proliferating at a normal rate, and the whole cell cycle is paused (Schwarzbacherová, V., 2019 and Galdíková, M, 2019). In addition to this pause in the cell cycle, an increase in lymphocyte chromosomal aberrations, sister chromatin exchange, and micronuclei, which are all signs of DNA damage, have been observed in human cells (Galdíková, M, 2019).

Along with ROS initiating apoptosis and potential DNA damage, Thiacloprid also plays a role in reproductive and neurological toxicity as well. As stated with other neonicotinoids, there are changes in male rats' fertility seen in sperm motility, sperm abundance, DNA fragmentation, and an increase in lipid and protein oxidation in the testicles when exposed to Thiacloprid (Kammoun, I., 2017). Additionally, glutathione, an intracellular scavenger of ROS to maintain homeostasis, was also decreased in the testicles, confirming lipid peroxidation and potential DNA damage (Kammoun, I., 2017). Thiacloprid impacts the ability to turn the gametes into blastocytes and significantly reduces blastocyte survivability (Babeľová, J., 2017). Negative fetal neurological developments have been associated with Thiacloprid as well. A study done in chicken embryos discovered that Thiacloprid induces ROS which increased malondialdehyde (MDA), a sign of carbonyl stress. MDA in addition with DNA damage found in the brain will negatively impact the brain's development (Farag, M, 2021). Additionally, Thiacloprid negatively impacts the Thyroid Hormone (TH), which can have a dangerous impact on fetal development including a lower IQ (Leemans, M., 2019).

Thiacloprid is also labeled by the EPA as a possible human carcinogen because female rats showed signs of reproductive carcinogenesis by developing uterine and ovarian cancer (EPA n.d.). Although there is little research into how the compound's metabolites cause tumorigenesis, it is suggested by several scientists that the compound dysregulates hormones and the cell cycle while creating reactive oxygen species which may lead to carcinogenesis (Galdíková, M, 2019, Şenyildiz, M., 2018, Schwarzbacherová, V., 2019). Hormone dysregulation and cell cycling disruption leads to the downregulation of CDK2, which can induce DNA damage (Schwarzbacherová, V., 2019). It is also possible that disformed blastocyte formation may also play a role in tumorigenesis as well (Babeľová, J., 2017).

Thiacloprid is also associated with thyroid cancer. Once ingested, the compound's metabolites will downregulate the thyroid stimulating hormone (TSH) cascading into lower iodine uptake, which causes Thyroxine (T4) and Triiodothyronine (T3) to be downregulated leading to higher amounts of monoiodotyrosine and diiodotyrosine (MIT and DIT) in the area due to no feedback signaling from the T3. As hormone dysregulation occurs, ROS is being created due to the Thiacloprid binding to the T3 (Leemans, M., 2019). With more ROS and growth hormone in the thyroid, it can cause tumor growth within the thyroid itself by creating an extra wall of cells around the existing thyroid to create a pocket where the tumor begins to grow (*What is Thyroid Cancer*, 2019).

Thiamethoxam:

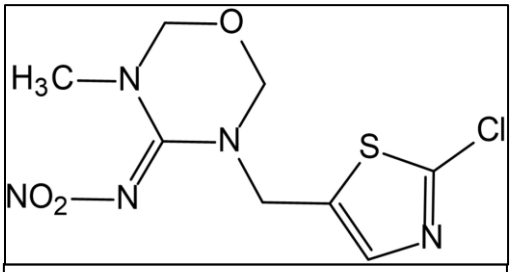


Figure 6. The chemical structure of Thiamethoxam.

Thiamethoxam (Figure 6), also known as Cruiser or Helix, is a neonicotinoid that is used in soil applications and seed treatment for alfalfa, cereal grains (including barley, buckwheat, corn, pearl millet, proso millet, oats, popcorn, rice (dry-seeded), rye, sorghum, teosinte, triticale, wheat and wild rice), cotton, cucurbit vegetables, legume vegetables (including soybean), oilseed crops (black mustard seed, borage seed, crambe seed, field mustard seed, flax seed, Indian mustard seed, Indian rapeseed seed, rapeseed seed, and safflower seed), peanuts, potatoes, sugarbeets, and sunflower. The half-life of the compound is between 3 hours in light and aqueous conditions or 15-22 days in soil (EPA n.d.). Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid. Thiamethoxan can be go through several nitro-reduction metabolic pathways to

form metabolites. This compound is associated with reproductive toxicity, cardiotoxicity, and is also a possible human carcinogen (Lui, Y, 2021, Feki, A., 2019, and El Okle, O.S., 2018).

Thiamethoxan is associated with reproductive toxicity by affecting follicle maturation and oocyte development. The compound reduces estrogen production in the granulosa cells and lowers follicle stimulating hormone receptor expression ultimately leading to a blockage of follicle development (Lui, Y, 2021). Without the follicle growth and granulosa cells proliferating, there is no oocyte development, maturation, or ovulation (Lui, Y, 2021). High concentrations of Thiamethoxam can trigger double strand breaks and other DNA damage in oocyte formation because of the high quantity of apoptosis induced (Lui, Y, 2021 and Babeľová, J., 2017). There are also mitochondrial clusters and vacuolation seen with Thiamethoxam which impairs the mitochondrial structure and can further impair oocyte formation (Lui, Y, 2021).

Thiamethoxam can also induce cardiotoxicity. Because Thiamethoxam can induce ROS formation, it has a very negative effect on the heart because it oxidizes the cardiomyocytes and upregulates stress-mediated apoptosis quickly (Feki, A., 2019). Thiamethoxam not only can kill off a vast amount of cardiomyocytes because of ROS, but the compound can also create cardiac membrane permeability, which causes enzyme leakage, which can lead to myocardial damage or death (Rajadurai, M, 2007, Feki, A., 2019). The compound can also induce high levels of lipids forming atherosclerosis which can lead to myocardial infarction (Feki, A., 2019).

Thiamethoxam is labeled a possible carcinogen because it may cause liver cancer. Many animal studies have shown that the growths that occur are non-mutagenic and rather the toxic effects mimic severe cirrhosis disease which then quickly devolves into liver cancer (El Okle, O.S., 2018). Some have shown that the compound does not form any liver cancer in rats (Green, T., 2005). However, mouse, rabbit, and fish models all have proven liver cancer can develop. In

many of the animal models, carcinogenesis can be seen with higher glucose levels, several pathological lesions, focal necrosis, fibrosis, inflammatory infiltration, and fatty infiltration (Khaldoun-Oularbi, H., 2021, and El Okle, O.S., 2018). Indication of severe damage is seen with a decrease in enzymic activities of ALT, AST, ALP, LDH, and GGT, which is accompanied by an increase in bilirubin (El Okle, O.S., 2018). Along with enzymes being decreased, there is also a down regulation of Tumor Necrosis Factor Alpha (TNFα) which inhibits apoptosis and may be potentially pro-carcinogenic (El Okle, O.S., 2018).

Clothianidin:

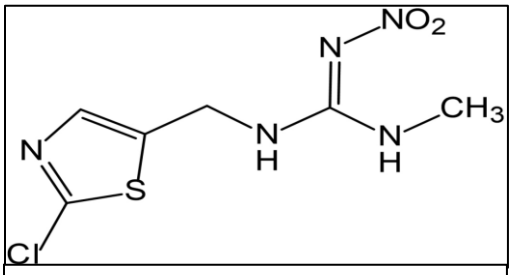


Figure 7. The chemical structure of Clothianidin.

Clothianidin (Figure 7), also known as Poncho 600, is a metabolite from another neonicotinoid, Thiamethoxam, and used as a seed treatment for corn and canola crops. The compound is stable in water and will break down within a day when exposed to light; while in soil, its half-life depends on light and anaerobic conditions which could break down between 34-148 days (EPA n.d.). Table 1 summarizes the half-life, LD50, and all crops approved for application of the neonicotinoid. Due to the structure of this compound, many of its metabolites that occur from ingestion or inhalation have been repeatedly shown to be noncarcinogenic in humans. Clothianidin can go through several chemical processes such as demethylation, de-nitration, urea formation hydrolysis, and glutathione conjugation to be excreted out of the body without accumulation (Atlı Şekeroğlu, Z., 2020). However, it does not mean that the compound does not bind to the nAChRs. One study has shown that Clothianidin can bind to the human nAChR, but it has a weak binding interaction.

However, once bound to the receptor it has a high efficacy to cause the receptor to stay activated (Li, P., 2011). Interestingly, Clothianidin is not dose respondent because once it reaches the highest point of activation the efficacy plateaus (Li, P., 2011).

Along with common neonicotinoid poisoning effects, there are animal studies that show that Clothianidin may cause reproductive issues and cytotoxicity as well (Bal, R., 2013). Much like acetamiprid, there are fertility troubles with sperm motility and concentration. Additionally, significant fatty acid composition builds up around the testes when rats are exposed to Clothianidin (Bal, R., 2013). In another study, Clothianidin was shown to cross over from mother to fetus via blood and that even at the acceptable daily limit there showed signs of mild neurobehavioral effects in adult mice, which had a worsened effect on the fetuses (Ohno, S., 2020).

Moreover, there are some effects that can only be seen with high dose exposures such as heightened immune response down regulating TNFα and NFκB and increased phosphorylation of ERK from nAChRs-mediated calcium signaling effecting apoptosis and cell proliferation, which leads to cytotoxicity (Di Prisco, G., 2017 and Hirano, T., 2019). However, there is conflicting research that suggests that under high concentrations Clothianidin will not induce cytotoxicity and genotoxicity because the compound breaks down into various metabolites before it can induce such forms of toxicity (Atlı Şekeroğlu, Z., 2020). However, it is shown that there is an impact in the lipid metabolism pathway in the liver, kidney, and colon when Clothianidin is ingested, but it is not significant to induce cytotoxicity (Atlı Şekeroğlu, Z., 2020).

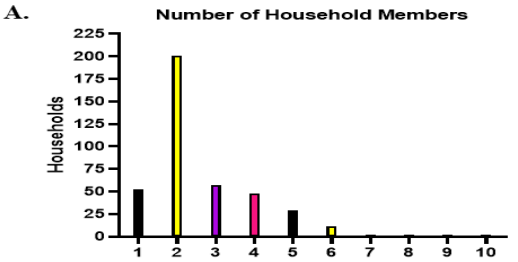
Compound	Half-Life	Ld50	Crops
Acetamiprid	Male Rats: 5.56 +/- 1.93h Female Rats: 4.42 +/- 1.10h 34 Days in Light and Aqueous Conditions 8-9 Days in Aerobic Soil	290 Mg/M3 (Rats)	Cotton, Leafy Vegetables, Cole Crops, Citrus Fruits, Pome Fruits, Grapes, Flowers, and Ornamental Plants
Clothianidin	Male and Female Rats: 2.9-4h 34-148 Days Depending on Light and Anaerobic Conditions	>5000 Mg/Kg (Rats)	Corn, Rapeseed, and Canola Crops
Dinotefuran	1.8 Days in Light and Aqueous Conditions 50-100 Days in Anaerobic Soil	2,804 Mg/Kg (Rats)	Leafy Vegetables, Ornamental Plants, and Lawn Care
Imidacloprid	1h in Light and Aqueous Conditions 40-126 Days in Anaerobic Soil	450 Mg/Kg (Rats)	Turfgrass (Including Sod Farms), Landscape Ornamentals, Fruit, and Nut Trees
Thiacloprid	10 Days- 1 Year in Aqueous Conditions 5-27 Days in Anerobic Soil	836 Mg/Kg (Rats)	Cotton and Pome Fruits
Thiamethoxam	3 Hours in Light and Aqueous Conditions 15-22 Days in Anerobic Soil	1,563 Mg/Kg (Rats)	Seed Treatments on Alfalfa, Cereal Grains, Cotton, Cucurbit Vegetables, Legume Vegetables, Oil Seed, Peanuts, Potatoes, Sugarbeets, and Sunflowers

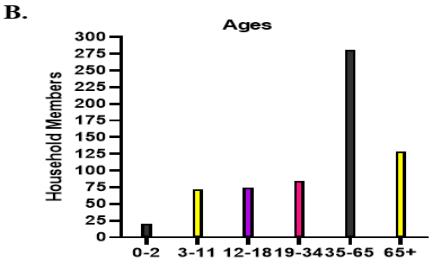
Table 1. Displays each compound's half-life, the median lethal dose (LD50) found in male rats, and crops that the compounds are approved for.

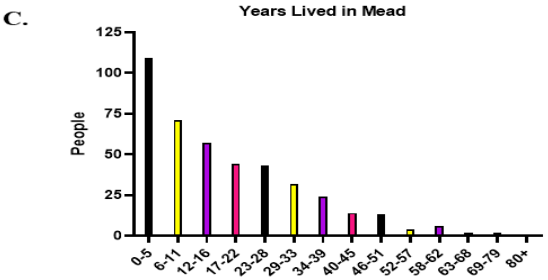
Neonicotinoid Exposure Concern in Mead, NE:

Mead, Nebraska, a small agricultural community consisting of approximately 626 people, was accidentally exposed to high amounts of neonicotinoids when AltEn, an ethanol plant that used neonicotinoid coated corn and soybean seeds for biofuel, was discovered not disposing of the toxic byproduct safely. Coincidentally, there was also a leak in their wastewater system that also contaminated the nearby areas. In the spring of 2022, UNMC sent out an Environmental Health Risk Perceptions Survey to 977 households and received 459 responses back. Many of the questions pertained to the demographics of the households as well as some mild exposure self-reporting questions. Some demographics obtained included how long they have lived in the house they currently are in, the ages seen in the households, and if the house was a multi-person inhabiting home. Figure 8 has details on demographics. Self-reporting questions addressed if they or anyone in the household experienced any health issues related to the exposure incident, whether anyone in the household has a chronic condition, mental health status, and stress index. Figure 9 has details on the self-reporting questions.

Based on this household survey, there is special interest of those who did notice health issued related to the incident because several of their health issues have been seen in both acute and chronic neonicotinoid exposures. As shown throughout this review, there is a cardiotoxicity, renal toxicity and possible carcinogenesis, reproductive toxicity and carcinogenesis, along with altered neurological conditions. As seen in many of the acute exposures there is also negative respiratory issues as well, which may play a role as to why so many people reported having respiratory problems.



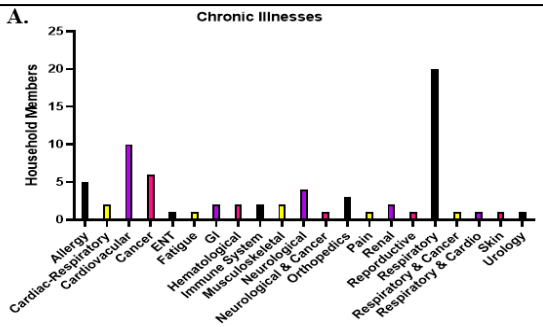


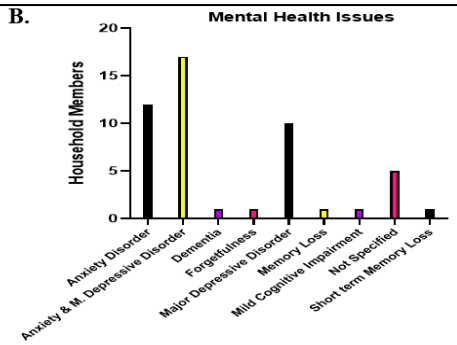


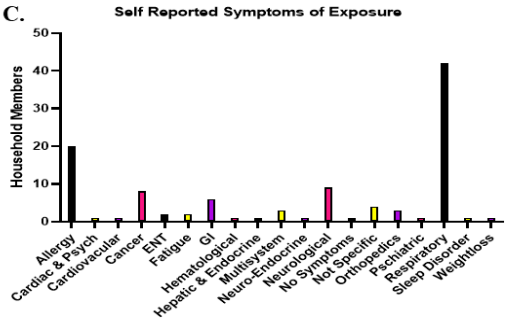
D.

Water source	Yes % (N)
Filtered municipal (tap)	23.31 (107)
Unfiltered municipal (tap)	21.13 (97)
Bottled water	45.10 (207)
Private well	55.34 (254)
Water from home distributors	8.93 (41)
Other	1.96 (9)

Figure 8. A.) Shows 403 responses to the question: "How many people live in your household?" B.) Shows 659 responses to the question: "What are the ages in your household?" The increase in response to the question is due to reporting all the ages within the household and not just the age of the person answering the survey. C.) Shows 422 responses to the question: "How many years has members of your household lived in Mead, NE?" D.)







D.

Issue	No stress	Some Stress	A Lot of Stress
Compromised health	28.76% 132	41.39% 190	16.78% 77
Feeling overlooked by decision makers	22% 101	33.33% 153	31.37% 144
Financial worry	43.14% 198	31.59% 145	11.11% 51
Feeling crisis will never be fixed	14.60% 67	33.99% 156	38.56% 177
Other	11.55% 53	3.92% 18	11.11% 51

Figure 9. A.) Shows 69 responses to the question: "Since 2015, what chronic illnesses have been diagnosed within your household?" B.) Shows 49 responses to the question: "What kind of mental health issues has your household members been experiencing?" C.) Shows 108 responses to the question: "Since 2015, what type of symptoms have you experienced that you feel are related to the activities of AltEn Plant?" AltEn is the abbreviation of the seed manufacturing factory that was involved in the wastewater contamination. D.) Shows the results to the question: "How much stress does your household experience related to the following issues due to the AltEn situation?" AltEn situation here is referring to the wastewater contamination discovery and clean up.

Moreover, neonicotinoids are also water soluble, and much of the area relies on well water or tap water, so there is potential for chronic neonicotinoid exposure from the drinking water and soil because neonicotinoids still may leech into the water as they work their way down through the soil and ultimately end up in the Ogalala aquifer. Many waste management systems do not measure neonicotinoids in the water and specifically filter them out. Although the water treatment facilities do chemically treat the water with various chemicals to lower bacterial contamination and ensure the water quality is up to EPA standards, neonicotinoid metabolites could potentially go through the treatment process and still end up in the consumed water supply. Additionally, because the plant was using neonicotinoid treated corn and soybean seeds, when the company permanently closed, the toxic piles of the byproduct as well as excess seed piles have been left outside on the facility's ground. This means that not only is the ground absorbing the byproduct and neonicotinoids from the seeds, but the rain and wind can also spread the neonicotinoids from just natural degradation of the seeds and byproduct.

While there is a clean-up effort and several ecological concerns for Mead after the AltEn event, it may take a few years to see just how much of an impact it has had on the community's overall health.

World Impact:

Currently researchers have been investigating population statistics of neonicotinoid metabolites found in urine. Because neonicotinoids are water soluble and many of the metabolites can clear the body via urine excretion, several studies have found that children ages 0-5/6 have the highest amounts of neonicotinoid metabolites in their bodies (Laubscher, B., 2019, Ospina, M. et al., 2019, Harada, K. H., 2016). This is believed because they are exposed to higher amounts of fresh produce in their diets. Another study found that mothers with higher amounts of neonicotinoids had babies with smaller cranial circumferences (Pan, C., 2022). Unfortunately, there has not been a follow up with that study to see if the decrease in size impacted neurological development as they predicted.

The Centers of Disease Control (CDC) discovered that neonicotinoids are higher in biological samples taken during the summer, than in the winter. The higher amount in summer could be due to more application of neonicotinoids for pests because it is growing season and insects are present whereas in the winter many of the produce comes from greenhouses (Ospina, M. et al., 2019). The CDC also noted higher neonicotinoid metabolites in people of Asian ethnicities than non-Asian ethnicities (Ospina, M. et al., 2019). They speculate that the higher amount in Asian ethnicities was due to high soybean diets and tea (Ospina, M. et al., 2019). While that speculation may not apply to all Asian ethnic groups, other researchers did discover that neonicotinoids can leech into brewed tea from tea crops. While the amount found in the green tea after brewing was below the daily dose limit of Dinotefuran (Bai, A. et al., 2021), there may be other sources of neonicotinoids in a person's diet which can build up in the body.

Globally, the EU is the only region to have a ban on three of the neonicotinoids: Imidacloprid, Thiamethoxam, and Clothianidin. While neonicotinoids are not as acutely toxic as

organophosphates, there are several different health risks that are associated with each neonicotinoid from infertility to cardiac problems. Yet, the much of the world is still using neonicotinoids on many commercialized crops. While the EPA does have application safety limits, there is little to no data on how much of the neonicotinoids are being absorbed into the actual produce that gets sold to the consumer. Each neonicotinoid has a different half-life which is dependent on conditions it absorbs into; therefore, the rate of decay could last from 1 day to 100+ days. It is unclear and largely unknown about the rate of decay for neonicotinoids absorbed into produce. Multiple applications and combination of different neonicotinoids can also impact the concentration and neonicotinoid metabolites found in the produce. This adds to the health risk because combinations of neonicotinoids can have a synergistic effect on the biological system (Alarcan, J., 2020). Furthermore, there are very few studies that have investigated if the produce interacts with the neonicotinoid and creates different metabolites that could cause a further toxicity when ingested by animals. Overall, there needs to be more research looking into the biological accumulation and the produce absorption and transference.

Conclusion:

Neonicotinoids are a classification of insecticides that strongly bind to and activate the nicotinic acetylcholine receptors (nAChR) and are unable to be inactivated by acetylcholine esterase (AChE) due to the chemical structure (Buszewski, B., 2019). This forces the central nervous system to be overstimulated, which can have numerous effects on the whole body because nAChRs are not only found in the neuromuscular but also the reproductive system (Phogat, A., 2022). Within the last 15 years, many researchers have discovered potential human toxicities associated with neonicotinoids, such as reproductive toxicity, rental toxicity, cardiotoxicity, cytotoxicity, and genotoxicity. Some neonicotinoids have been shown to be a possible human carcinogen like thiacloprid causing thyroid and ovarian cancer and thiamethoxam causing liver cancer.

The gap in research begins with not knowing the total amount of neonicotinoids consumers are ingesting from neonicotinoid treated crops. The gap then leads into defining a new threshold limit because there are many lifestyle factors that can impact the neonicotinoid biological response such as smoking and drinking which also induce lipid peroxidation. While the EPA does currently have LD50 threshold limits seen in Table 1, the limits are specific to that compound only. The EPA did not investigate any synergistic effect that could occur with different crops being treated with different neonicotinoids. However, it's clear that consumers are ingesting more than one neonicotinoid from many different crops and there are studies showing that there is accumulation of the neonicotinoids in the human body affecting our human health as seen with mothers birthing children with smaller cranial circumferences (Villar, J., 2021).

Having significant amounts of research showing the potential human health effects from acute and chronic neonicotinoid exposure is very concerning for the people of Mead, who have

been exposed to high amounts (and still are being exposed to neonicotinoids) as the clean-up efforts are underway. The lasting effects on human health in their community will take a few years before being seen. As with most chronic diseases and carcinogens, they are slow developing, and may not cause any immediate health problems. Those that may see more of an impact to their health are children, teens, and adults of childbearing age because there is neurological developmental problems and reproductive issues, such as infertility, that can occur with chronic and acute exposure to neonicotinoids. Children might be impacted more due to their high rate of neonicotinoid exposure in their diet already (Ospina, M. et al., 2019).

Overall, neonicotinoids have been used in agriculture for over two decades. Because these compounds are new, many coming into market in the early 2000's, researchers are just now discovering the negative human health impacts these compounds can have. While there is still a lot that remains unknown, by compiling some of the major effects that neonicotinoids present in research studies, it may help give a better insight as to how serious these compounds are and where more research is needed.

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