Copper Products (Fixed Coppers and Copper Sulfate)

Crops

Identification of Petitioned Substance

Chemical Names:
copper(II) sulfate (anhydrous); copper(II) sulfate pentahydrate; copper(II) sulfate basic; copper(II) hydroxide; copper(II) oxide; copper(I) oxide (cuprous oxide); copper(II) oxychloride; copper(II) oxychloride sulfate; copper(II) carbonate hydroxide; copper(II) ammonia complex; copper(II) ethylenediamine complex; copper salts of fatty acids and rosin acids; copper(II) octanoate

Trade Names:
Bonide Copper Spray or Dust; Drexel Basic Copper Sulfate; Blue Shield DF; Kocide 2000; Nu-Cop XLR; Spinnaker; Badge SC; plus many others. Refer to the National Pesticide Information Center's website tool, NPRO: http://npic.orst.edu/NPRO/

Copper Sulfate Crystals; Basic Copper 53; OB Old Bridge Chemicals Copper Sulfate Fine Crystals; Nu-Cop® 50DF Fungicide/Bactericide; Technical Copper Oxychloride; COP-KLEEN Algicide/ Bactericide/ Fungicide; Econatur FUNGICAR Fungicida Liquido Soluble

Other Names:
cupric sulfate; cupric sulfate; bluestone; blue vitriol; chalcanthite; tribasic copper sulfate; brochantite; cupric hydroxide; copper dihydroxide; cupric oxide; copper oxide black; tenorite; cuprous oxide; Copper oxide red; dicopper oxide; cuprite; dicopper chloride trihydroxide; basic copper chloride; basic copper carbonate; cupric carbonate; malachite; tetraamminecopper(II) ion; copper ammine complex; cupriethylenediamine; copper 2-azanidylethylazanide; copper caprylate; copper soap; octanoic acid, copper salt

CAS Numbers:
7758-98-7; 7758-99-8; 1344-73-6; 20427-59-2; 1317-38-0; 1344-70-3; 1317-39-1; 1332-65-6; 12069-69-1; 16828-95-8; 13426-91-0; 20543-04-8

Other Codes:
EPA PC Code: 024408; 024401; 008101; 023401; 042401; 025601; 023501; 022901; 022702; 024407; 023306

FDA UNII: KUW2Q3U1W; LRX7AJ16DT; 3314XO9W9A; V1XJQ704R4; T8BEA5064F; 76712031PG; GIK928GH0Y; J68H5PUV30; NIP4I4LVCC

Summary of Petitioned Use

The National List of Allowed and Prohibited Substances at 7 CFR 205.601(i) has included coppers, fixed and copper sulfate *as plant disease control*, since its inception (65 FR 80547). Currently, these appear on the National List in § 205.601, *Synthetic Substances Allowed for Use in Organic Crop Production* as follows:

(i) As plant disease control

(2) Coppers, fixed - copper hydroxide, copper oxide, copper oxychloride, includes products exempted from EPA tolerance, Provided, That, copper-based materials must be used in a manner that minimizes accumulation in the soil and shall not be used as herbicides.

(3) Copper sulfate - Substance must be used in a manner that minimizes accumulation of copper in the soil.

In 2003, the National Organic Program (NOP) added two additional uses for copper sulfate: to § 205.601(a) for use as an algicide, and in § 205.601(e) for use as an insecticide, to control tadpole shrimp[1] (68 FR 61987).

(a) As algicide, disinfectants, and sanitizer, including irrigation system cleaning systems

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[1] Tadpole shrimp are technically crustaceans, not insects.

(3) Copper sulfate - for use as an algicide in aquatic rice systems, is limited to one application per field during any 24-month period. Application rates are limited to those which do not increase baseline soil test values for copper over a timeframe agreed upon by the producer and accredited certifying agent.

(e) As insecticides (including acaricides or mite control)

(4) Copper sulfate - for use as tadpole shrimp control in aquatic rice production, is limited to one application per field during any 24-month period. Application rates are limited to levels which do not increase baseline soil test values for copper over a timeframe agreed upon by the producer and accredited certifying agent.

In the fall of 2021, the National Organic Standards Board (NOSB) recommended that the algicide and insecticide listings for copper sulfate be renewed as part of the 2023 Sunset Review Process (NOSB, 2021). These uses are listed at §§ 205.601(a) and 205.601(e). However, plant disease control uses at § 205.601(i) were outside the scope of the NOSB's 2021 recommendation.

This TR supports the NOSB's review of coppers, fixed, and copper sulfate for use as plant disease control, under § 205.601(i). As such, this report will not focus on uses related to §§ 205.601(a) and 205.601(e).

Where possible, this report includes information on the different forms of fixed coppers and copper sulfate. However, there are sections of this report which do not include information on each form, either because of insufficient literature, or because considering all forms would be impractical in a single technical report.

Characterization of Petitioned Substance

Composition of the Substance:

Copper products include 12 materials, some of which could themselves include more than one substance. These materials are described below.

Copper(II) sulfate (includes anhydrous copper(II) sulfate, copper(II) sulfate monohydrate, copper(II) sulfate trihydrate, and copper(II) sulfate pentahydrate) :

Copper(II) sulfate is a mineral salt that occurs in multiple hydration states[2] with the simplified formula $CuSO_4 \cdot nH_2O$ (where n represents variable hydration states). Depending on atmospheric conditions, water is incorporated into the crystal structure in different proportions (Richardson, 2000). Anhydrous copper(II) sulfate contains no water. Copper(II) sulfate monohydrate contains one incorporated water molecule, trihydrate contains three water molecules, and pentahydrate contains five water molecules. Copper(II) sulfate pentahydrate is by far the most widely used copper(II) sulfate hydrate because of production and storage parameters (Richardson, 2000). Because of its prevalence, copper(II) sulfate pentahydrate is often referred to simply as "copper sulfate," and sometimes as "bluestone." Unless otherwise specified, the widely used copper(II) sulfate pentahydrate found in the vast majority of agricultural fungicide and bactericide copper sulfate products will be referred to as copper(II) sulfate in this report.

Copper(II) sulfate basic:

Copper(II) sulfate basic may refer to several different compounds, containing variable amounts of water or copper hydroxide in their structures. The CAS No. 1344-73-6 appearing on *Copper; exemption from the requirement of a tolerance* at 40 CFR 180.1021 refers to the compound incorporating three copper hydroxide molecules, $CuSO_4 \cdot 3Cu(OH)_2$, sometimes referred to as "tribasic copper sulfate." Naturally occurring tribasic copper sulfate is known as the mineral brochantite (CAS No. 12068-81-4), a fairly common result of the weathering of copper. Richardson (2000) states that tribasic copper sulfate is the most commercially prevalent copper(II) sulfate basic compound.

Copper(II) hydroxide:

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[2] Hydration state refers to the amount of water that is part of the crystal structure of a substance. Minerals can often shift between hydration states through physical processes, such as heating, drying, or through exposure to water or humid air.

Copper(II) hydroxide, $Cu(OH)_2$, is a weak base that sparingly dissociates into Cu^{2+} and hydroxide (OH^{-}) ions in aqueous solution. It can decompose to copper(II) oxide on contact with air and heat, and may be coated with gelatin to enhance stability (Richardson, 2000).

Copper(II) oxide:
Copper(II) oxide, also known as "cupric oxide," or "copper oxide black," has the molecular formula CuO. Copper(II) oxide occurs naturally in oxidized zones of copper ore deposits as the mineral tenorite (Wisconsin Geological and Natural History Survey, 2020).

Cuprous oxide:
Cuprous oxide, copper(I) oxide, or "copper oxide red" has the molecular formula Cu_2O and differs from copper(II) oxide by the oxidation state of the copper atom. Cuprous oxide is the only compound included in this report with a Cu^{+} (cuprous) ion instead of a Cu^{2+} (cupric) ion. Naturally occurring cuprous oxide occurs as the mineral cuprite (Patnaik, 2003).

Copper(II) oxychloride
Sometimes referred to as "basic copper chloride" or "dicopper chloride trihydroxide," copper(II) oxychloride consists of copper(II) chloride salt mixed with copper(II) hydroxide, with the molecular formula $Cu_2Cl(OH)_3$ (Richardson, 2000).

Copper(II) oxychloride sulfate
Copper(II) oxychloride sulfate is a mixture of the two active ingredients: copper(II) oxychloride and copper(II) sulfate basic (EPA, n.d.).

Copper(II) carbonate hydroxide
Copper(II) carbonate hydroxide, also known as "basic copper carbonate," has the molecular formula $CuCO_3 \cdot Cu(OH)_2$, sometimes written as $Cu_2CO_3(OH)_2$.

There may be an inconsistency with the CAS number listed at 40 CFR 180.1021 (*Copper; exemption from the requirements of a tolerance*). Basic copper carbonate (malachite) is identified with the CAS number 1184-64-1, but this CAS number identifies neutral or pure copper carbonate ($CuCO_3$), a material not found in nature and only synthesized in small quantities under experimentally difficult high-pressure conditions (Gamsjager & Preis, 1999; Richardson, 2000). Additionally, there appears to be no product registered with the U.S. Environmental Protection Agency (EPA) under the CAS number 1184-64-1, but several with the CAS number 12069-69-1 (EPA, 2016a). Lamichhane et al. (2018) states that basic copper carbonate, CAS number 12069-69-1, is a commonly used copper compound for foliar disease management. During the writing of this report, we reached out to the EPA for comment, but did not receive clarification.

Copper(II) ammonia complex
The CAS number 16828-95-8, described at 40 CFR 180.1021, refers to the tetraamminecopper ion with the molecular formula $Cu(NH_3)_4^{2+}$. The tetraammine complex (containing four ammonia molecules) tends to predominate in solutions with low ammonia concentrations (Richardson, 2000). Though the methods of preparation are complex, the simplified trend is that increasing the ammonia concentration increases the number of ammonia ligands per copper ion, forming different amine copper complexes (mono, di, tetra, penta, hexa, and deca) (Hathaway & Tomlinson, 1970). Diamminecopper forms are favored in solutions containing copper(I) ions (Richardson, 2000).

Copper(II) ethylenediamine complex
Ethylenediamine ($C_2H_8N_2$) is a colorless liquid with an ammonia odor comprising a two carbon chain with amino groups (NH_2) at either end (PubChem, 2022) that forms a very stable five member ring chelate with the transition metals including the copper(II) ion. The ethylenediamine chain bends around itself so both ends bond to the copper ion, forming a copper, carbon, and nitrogen ring.

Copper salts of fatty acids and rosin acids

Fatty acids are chains of carbon atoms that may be saturated[3] or unsaturated, containing a carboxylic acid (COOH) group at one end. Over 1000 fatty acids are known, but only 20-50 are commonly used; some common examples include lauric, myristic, palmitic, stearic, oleic, erucic, and linoleic, all derived from vegetable or seed oils (Gunstone, 2012). Copper bonds to the carboxylic end of fatty acids to form a salt, and these compounds are commonly known as "soap salts" (National Pesticide Information Center, 2001). Copper octanoate is one of the copper salts of fatty acids (NOP, 2011).

Rosin acids, often derived from plant material (pine trees), are a group of chemicals containing three hydrocarbon rings and a carboxylic acid functional group (Brocas et al., 2014). Like the copper salts of fatty acids, copper bonds to the carboxylic acid functional group.

Copper(II) octanoate

Copper(II) octanoate ($C_{16}H_{30}CuO_4$) is one of many copper salts of fatty acids. Octanoic acid (sometimes known as caprylic acid) is a saturated medium-chain fatty acid consisting of eight carbon atoms and a carboxylic acid at one end, usually derived from palm kernel oil (Stamatopoulou et al., 2020). Octanoate is the conjugate base of octanoic acid (the ion that forms when an acid loses the acidic hydrogen ion). The positively charged copper ion bonds to two negatively charged octanoate ions at their carboxylic acid functional groups to form the copper(II) octanoate fatty acid salt, sometimes referred to as copper caprylate or simply copper soap.

Source or Origin of the Substance:

The Earth's crust contains approximately 50-60 parts per million (ppm) copper, as a global average (Lossin, 2001; Press & Siever, 1986). The concentration can be significantly elevated in volcanic and hydrothermal rocks, most often as sulfide or oxide minerals. These deposits are the primary sources of copper used in all industries (Lossin, 2001; Press & Siever, 1986). In soils, background copper ranges in concentration from 2-50 ppm depending on the geologic environment (Alloway, 2013). The average copper concentration is typically less than 1 ppm in seawater (Lossin, 2001; World Health Organization, 2004), and less than 10 ppm in freshwater (Drever, 1997).

Commercially important copper ore deposits are all formed as a result of some type of volcanic activity (Lossin, 2001). The geologic processes involved in the formation of copper ore deposits include (Duran et al., 2017; Hitzman et al., 2010; Lossin, 2001):

- Chemical alteration of deep volcanic rocks by hot acidic fluids (hydrothermal alteration)
- Infiltration of volcanic fluids or thermal springs into sedimentary layers associated with continental rift zones
- Density separation in molten magma bodies
- Direct crystallization of copper-bearing minerals in slowly cooling magma[4]

Of these deposit types, the first two are by far the most commercially important sources, making up an estimated 80% of identified copper resources (Pietrzyk & Tora, 2018). In terms of mineral type, copper sulfide minerals make up 90% of recoverable copper, and copper oxides account for 9% (Pietrzyk & Tora, 2018).

Copper production exists on every continent except Antarctica (Lossin, 2001). Though not a current producer, Antarctica may become an important source of copper in the future (Lossin, 2001). As of 2016, Chile and Peru are the largest copper producing countries in the world, followed by Congo, China and the United States (Pietrzyk & Tora, 2018; U.S. Geological Survey, 2022).

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[3] Saturation refers to the degree to which hydrogen is bonded to carbon atoms. A fully "saturated" fatty acid will contain only single bonds between carbon atoms, and 2 hydrogen atoms per carbon (except at the ends, where carbon can be bonded to three hydrogen atoms). An unsaturated fatty acid will have at least one double bonded carbon pair, with only 1 hydrogen bonded. This often creates a "kink" in the fatty acid structure.

[4] As magma crystallizes deep in the Earth, different minerals crystallize at different temperatures and pressures, leading to distinct zones of mineral groups and thus, the possibility of areas with elevated copper. This is known as fractional crystallization (Duran et al., 2017).

Higher grade copper ores (1% or greater copper) are sometimes mined underground, while lower grade deposits (approximately 0.5% copper) are exploited in open pit mines (Lossin, 2001). In general, ore is extracted by drilling, blasting, and hauling to processing plants. Though numerous techniques exist to purify copper ore, they can be categorized into two types, summarized here.

Pyrometallurgical processes
Copper ore is crushed and milled to separate individual minerals. The resulting milled rock is subjected to froth flotation in which water, air bubbles, and other chemicals are introduced, separating valuable minerals from waste rock by their specific surface properties through a process known as beneficiation (Lossin, 2001). The concentrated copper mixture enters high heat furnaces (smelters) to melt and separate copper/iron sulfides (matte) from waste silicates and metal oxides (slag) (Lossin, 2001; Pietrzyk & Tora, 2018). Following smelting, molten "matte" is oxidized to separate the copper and iron components, resulting in crude copper metal (98-99% purity) containing pockets of sulfur dioxide gas, known as "blister" (Lossin, 2001). Blister copper is further purified by melting in furnaces and then cast into slabs called anodes, which can be further refined using electrolysis processes, resulting in up to 99.99% pure copper metal (Lossin, 2001; Pietrzyk & Tora, 2018).

Hydrometallurgical processes
Processors may use hydrometallurgical (liquid extraction, a.k.a. leaching) methods on raw ore material, mine waste (tailings), or on pre-concentrated copper ore (Lossin, 2001). In the case of copper ore, the leaching solution is almost invariably sulfuric acid (Petersen, 2016).

Four primary leaching methods may be used (Petersen, 2016)

- *In situ* leaching—involves the application of a leaching solution to ore that remains in the ground. The solution percolates through the natural pores of the rock, or the rock is blasted or hydrofractured in order to open channels in the deposit.
- Dump leaching—involves spraying the leach solution onto piles of raw extracted ore and collecting the leachate from the bottom. Dump leaching is useful for very low-grade waste rock, and results in low extraction values because the ore has not yet been crushed.
- Heap leaching—essentially the same process as dump leaching but results in higher yields because ore is first pulverized. Heap leaching may be useful for intermediate-grade ores because it carries higher operating costs than dump leaching.
- Vat leaching—filling basins with pulverized ore and flooding with leach solution, after which the liquid is drained off.

Heap leaching with sulfuric acid is the most common hydrometallurgical extraction process used for copper oxide and copper sulfide ore (Petersen, 2016). The leaching period may range from four months to three years (Petersen, 2016). Sulfuric acid is sprayed on pulverized ore, dissolving copper that is then collected from the bottom of the heap (Lossin, 2001; Petersen, 2016).

In situ leaching may also be used, in which sulfuric acid is applied directly to the rock in place, but this is currently a minor practice for copper. Copper ores of igneous origin are not permeable enough for *in situ* leaching to be effective (De Silva et al., 2018). Hydraulic fracturing, combined with a decline in copper ore grades, may make *in situ* leaching more prevalent in the future for sediment hosted copper deposits, which make up approximately 25% of global copper ores (De Silva et al., 2018). The dissolved copper-rich acid is refined to copper metal by running electric current through the solution, depositing copper metal on cathodes for collection (a process known as "electrowinning") (Lossin, 2001).

Oxidizing bacteria also play a role in heap leaching of copper ores with high iron and sulfide content, as they oxidize ferrous (Fe^{2+}) iron to ferric (Fe^{3+}) iron, and sulfur compounds to sulfates (Petersen, 2016). The formation of ferric (Fe^{3+}) iron causes copper(I) sulfide minerals to dissolve (Petersen & Dixon, 2007). The copper^{1+} ion is subsequently oxidized to copper^{2+} and leaches out of the ore pile, where it is collected (Petersen & Dixon, 2007). Iron(III) sulfate forms, as well as additional sulfuric acid that increases leaching potential (Petersen & Dixon, 2007).

See *Evaluation question #6* for more information on environmental contamination due to copper mining processes.

Recycled copper is another significant source, and can be used as a starting material in either of the above described production processes. Waste materials derived from copper mining/refining, scrap from direct production of materials, and post-consumer scrap metal may be recycled (Pietrzyk & Tora, 2018). As of 2016, approximately 17% of refined copper was derived from recycled sources (Pietrzyk & Tora, 2018). Recycled copper from scrap made up 32% of United States copper supplies in 2021 (U.S. Geological Survey, 2022).

Approximately 70% of copper is used in electronic devices, electrical power generation/distribution, and communications (Pietrzyk & Tora, 2018). About 20% of copper is used in construction for pipes, roofs, or decorative panels. The remaining 10% is used in coins, jewelry, musical instruments, statuary, kitchen products, and other consumer goods (Pietrzyk & Tora, 2018). "Other consumer goods" includes copper compounds used in agriculture.

Specific production processes for the manufacture of copper compounds used as agricultural pesticides are described in *Evaluation question #2,* below.

Properties of the Substance:

See *Appendix A: Chemical and Physical Properties of Relevant Copper Compounds* for technical data regarding the copper compounds discussed in this report.

The copper compounds covered in this report are salts or complexes, but all ultimately break down into the active copper ion, most often the cupric form (Cu^{2+}) (Husak, 2015). Salts derived from copper(II) are typically blue or green (Lossin, 2001). The differing solubilities of the compounds determine their efficacy and the mobility of the active copper ion (Richardson, 1997). Copper(II) sulfate is the most soluble. The other compounds discussed here are considered "fixed copper" compounds characterized as sparingly soluble to practically insoluble (Richardson, 2000). The fixed coppers act as controlled release pesticides due to their limited solubility. The addition of adjuvants may influence the residence time on a plant's surface (Richardson, 1997). Particle size of the pesticide may also play a significant role in the mobility of the active component, since larger particles tend to break down and erode more slowly than smaller particles (Richardson, 1997).

In solution, copper typically occurs as the divalent cation (Cu^{2+}), either as a free ion or complexed with other substances (Drever, 1997; Richardson, 2000). In oxidizing conditions (as in aerobic soils), copper ions are relatively soluble and mobile at lower pH levels and less soluble with increasing pH as carbonate, oxide, and hydroxide compounds form (Drever, 1997). Divalent copper readily complexes with organic matter, often in the form of humic substances (Drever, 1997; Ponizovsky et al., 2006).

Copper is an essential element for all organisms, playing roles in enzyme formation, iron absorption, oxidation/reduction reactions, mitochondrial respiration, and many other biological processes, either as copper(II) or reduced copper(I) (Alloway, 2013; Tapiero et al., 2003). Excess copper may also injure cellular components; specific cellular proteins work to maintain the balance needed in certain tissues (Tapiero et al., 2003). For more information regarding copper regulation and the effects of copper on organisms, see *Evaluation Questions #5, 8,* and *10*; as well as *Focus Question #1*.

In plants, trace quantities of copper play a role in carbohydrate and protein metabolism along with other physiological processes (Rehman et al., 2019). However, copper may also inhibit photosynthesis in plant cells and is particularly toxic to algae (Husak, 2015). In bacteria, fungi, and mollusks copper binds to functional groups on proteins and enzymes, resulting in cellular damage and leakage across the cell membrane (Husak, 2015). Cupric (Cu^{2+}) and cuprous (Cu^{+}) ions participate in oxidation/reduction ("redox") reactions in animal cells which may form free radicals in the form of reactive oxygen species (ROS), leading to destruction of DNA and other molecules (Husak, 2015).

Specific Uses of the Substance:

As stated in *Summary of Petitioned Use* (above), fungal and bacterial disease control are the focal points of this report.

The NOP also permits the use of sulfates, carbonates, oxides, or silicates of copper as plant micronutrients in the case of a documented tissue or soil deficiency at 7 CFR 205.601(j)(7)(ii). Copper deficiency is most often associated with sandy soils or those with high contents of peat, which acts as a copper binding agent, reducing availability of copper to plants (Alloway, 2013). Wheat, alfalfa, and lettuce are prone to copper deficiency (Alloway, 2013).

Copper sulfate also appears on the National List at § 205.603(b)(1) for use as a topical treatment, external parasiticide, or local anesthetic for organic livestock production. Many copper compounds are also permitted as trace minerals used for enrichment or fortification in livestock feed, when Food and Drug Administration (FDA) approved, at § 205.603(d)(2). The FDA allows copper carbonate, copper chloride, copper gluconate, copper hydroxide, copper orthophosphate, copper oxide, copper pyrophosphate, and copper sulfate as trace minerals in animal feed at 21 CFR 582.80.

Agricultural uses:

Copper is used as a broad-spectrum fungicide, bactericide, herbicide, algaecide and molluscicide in agricultural settings, with EPA-registered uses in food, field and greenhouse crops, for aquatic applications, and residential homes (EPA, 2009).

The most commonly used fungicidal and bactericidal <u>fixed copper</u> compounds are copper(I) oxide, copper(II) oxychloride, copper(II) sulfate basic, and copper(II) hydroxide (Richardson, 1997).

In organic production, copper pesticides are particularly useful against fungal diseases such as downy mildew on grapes, late blight of potato, apple scab, various coffee diseases, and bacterial infections such as tomato spot, citrus canker, fire blight of pome fruit, walnut blight, stone fruit canker, apical necrosis in mango, and olive knot (Lamichhane et al., 2018). A survey of EPA-registered pesticide labels indicate use instructions and application rates on nearly every variety of commercial crop, including fruits, nuts, herbs, grains, vegetables, legumes, trees, greens, ornamentals, and grasses (EPA, 2009).

Copper pesticides are most often applied as a foliar spray (Husak, 2015), but copper formulations may be sold as dusts, liquid concentrates, dry flowable particles, wettable powders, granules, aerosols, and crystals (EPA, 2009).

Aquatic uses:

For aquatic applications, copper is EPA registered for numerous uses, including (EPA, 2009):

- Algae control in aquaculture facilities, drainage structures, ponds, fountains, lakes, irrigation systems, and sewage lagoons
- Weed control in aquaculture facilities, drainage structures, ponds, fountains, lakes, irrigation systems, and sewage lagoons
- Freshwater snail control
- Leech and tadpole shrimp control in rice fields

Of the copper compounds discussed in this report, copper(II) sulfate far outweighs the others in total use in aquatic environments, where high solubility is desired (EPA, 2009).

Other biocidal uses:

Copper compounds are also used outside of agriculture as biocides in wood preservatives, and as components of paints formulated to prevent accumulation of biological organisms,[5] notably on boat hulls (EPA, 2009). In the past decade, numerous studies have been conducted on the use of antibacterial copper oxide nanoparticle coatings on medical devices, touch surfaces, paints, and fabrics (Meghana et al., 2015).

Products approved for plant disease control:

At the time of this report, the Organic Materials Review Institute (OMRI) lists 131 copper products allowed for use in organic production for plant disease control (OMRI, 2022). Of these 131, 61 are EPA registered. Many others are marketed to countries outside the U.S., most notably in Latin America. OMRI groups these products in two categories within the Crop Pest, Weed and Disease Control (CP) classification:

- Copper sulfate
- Coppers, fixed

Copper(II) sulfate pentahydrate is included alone at 7 CFR 205.601(i)(3) of the National List, but is also included under § 205.601(i)(2) as a fixed copper with an EPA tolerance exemption. Therefore, copper(II) sulfate pentahydrate products appear in both OMRI categories.

Table 1 summarizes the OMRI Listed products by their active ingredients.

Table 1: OMRI Listed copper products grouped by active ingredient

Category	**Active Ingredient**	**Products**
Copper sulfate	copper(II) sulfate pentahydrate	50
	copper(II) sulfate basic	1
Coppers, fixed	copper(II) sulfate pentahydrate	15
	copper(II) sulfate basic	2
	copper(II) hydroxide	28
	copper(I) oxide	8
	copper(II) oxide	1
	copper(II) oxychloride	12
	copper(II) octanoate	9
	dual: anhydrous copper(II) sulfate/copper(II) oxychloride	1
	dual: copper(II) sulfate basic/copper(II) oxychloride	2
	dual: copper(II) hydroxide/copper(II) oxychloride	2

Approved Legal Uses of the Substance:

Environmental Protection Agency (EPA):

Copper is used both as an active pesticidal ingredient and as an inert ingredient. The EPA issued "Reregistration Eligibility Decision (RED) for Coppers – Revised May 2009" (EPA, 2009) to support the process of reregistration of copper-based pesticides. The report assessed the impacts of copper use on human health, as well as ecological risks to provide updated pesticide labeling and use standards. In addition to establishing registration requirements for copper-based pesticides, the report also determined the following "restricted-entry intervals" (the period of time for which a producer may not allow a worker to enter a treated area) for each substance (Table 2). The restricted-entry interval is determined by the acute toxicity of each copper compound, with longer intervals corresponding to higher toxicity levels (EPA, 2009).

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[5] Paints used to prevent the accumulation of biological organisms are known as "antifouling" paints, and work to reduce the ability of marine organisms (like barnacles, bacterial biofilms, or algae) to latch onto a boat's hull, thus increasing fuel efficiency and cost reduction (Chambers et al., 2006). There has been recent interest in reducing the use of copper-based antifouling coatings due the risk of biomagnification in the marine food chain (Chambers et al., 2006).

Table 2: Copper compounds and their associated restricted-entry interval. Adapted from U.S. EPA, 2009.

Copper Compound	**Restricted-entry Interval**
Copper(II) sulfate basic: (CAS. No. 1344-73-6)	48 hours
Copper(II) hydroxide: (CAS No. 20427-59-2)	48 hours
Copper(II) oxide: (CAS No. 1317-38-0, 1344-70-3)	12 hours
Cuprous oxide: (CAS No. 1317-39-1)	12 hours
Copper(II) oxychloride (CAS No. 1332-65-6)	48 hours
Copper(II) oxychloride sulfate (CAS No. 8012-69-9)	48 hours
Copper(II) ammonia complex (CAS No. 16828-95-8)	48 hours
Copper(II) ethylenediamine complex (CAS No. 13426-91-0)	24 hours
Copper salts of fatty acids and rosin acids	48 hours
Copper(II) octanoate (CAS No. 20543-04-8)	12 hours

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) governs the registration, use, and sales of pesticides in the U.S. (Federal Insecticide, Fungicide, and Rodenticide Act, 2017). FIFRA also includes a list of substances that are exempt from the requirement of the establishment of a tolerance. A "tolerance" is the maximum amount of pesticide residue above which there are demonstrated hazardous health impacts. Regulations at 40 CFR 180.1021 include the tolerance exemptions for copper residues on or in plant or animal commodities. Table 3 contains the relevant sections of 40 CFR 180.1021 for organic production. The exemptions in Table 3 indicates that the EPA does not believe that there is a residue level above which there will be negative impacts on human or animal food.

Table 3: 40 CFR 180.1021(b): "The following copper compounds are exempt from the requirement of a tolerance when applied (primarily) as a fungicide to growing crops using good agricultural practices."

Copper compounds	**CAS Reg. No.**
Basic copper carbonate (malachite)	1184-64-1
Copper ammonia complex	16828-95-8
Copper ethylenediamine complex	13426-91-0
Copper hydroxide	20427-59-2
Copper octanoate	20543-04-8
Copper oxychloride	1332-65-6
Copper oxychloride sulfate	8012-69-9
Copper salts of fatty and rosin acids	9007-39-0
Copper sulfate basic	1344-73-6
Copper sulfate pentahydrate	7758-99-8
Cuprous oxide	1317-19-1

Food and Drug Administration (FDA):

The FDA has approved copper for a variety of uses. Copper is allowed as a nutritive mineral at 21 CFR 101.9(c)(8)(iv) in the daily amounts listed in Table 4:

Table 4: Daily allowance of copper as a nutritive mineral at 21 CFR §101.9(c)(8)(iv)

Nutrient	**Adults and children ≥ 4 years**	**Infants[1] through 12 months**	**Children 1 through 3 years**	**Pregnant women and lactating women**
Copper	0.9 mg	0.2 mg	0.3 mg	1.3 mg

There are currently two FDA-approved drugs with copper as the active ingredient. One drug is a radioactive diagnostic agent (FDA, 2020), and the other is an intrauterine device used for birth control (FDA, 1984).

The FDA has also reviewed the following forms of copper as Generally Recognized as Safe (GRAS) materials.

- Copper gluconate (cupric gluconate ($CH_2OH(CHOH)_4COO)_2Cu$; CAS Reg. No. 527-09-3) is allowed as a nutrient supplement and synergist agent at 21 CFR 184.1260.
- Copper sulfate in the pentahydrate form is allowed as a nutritive supplement and processing aid at 21 CFR 184.1261.
- Cuprous iodide is allowed within table salt at 21 CFR 184.1265.

Several forms of copper are included as allowed color additives by the FDA (FDA, 2021). Metallic copper powder is allowed for use in externally applied drugs as well as in cosmetics, including for products used in the area of the eyes.

The FDA has also included a number of copper compounds as allowed for inclusion in animal feed. 21 CFR 582.80 lists the trace minerals that can be added to animal feeds, including:

- copper carbonate
- copper chloride
- copper gluconate
- copper hydroxide
- copper orthophosphate
- copper oxide
- copper pyrophosphate
- copper sulfate

Additionally, 21 CFR 582.5260 specifically limits copper gluconate to a level not exceeding 0.005%.

Action of the Substance:

Copper must be solubilized before fungi or bacteria respond to its toxicity (Richardson, 1997). The divalent, Cu^{2+}, form of copper is toxic to microorganisms, and the monovalent form, Cu^{+}, readily oxidizes to the divalent ion (Trevors & Cotter, 1990). The prevalence of complexing agents in the soil, such as amino acids, sugars, thiols, proteins, or certain organic acids tend to reduce the efficacy of copper bactericides by reducing the mobilization of dissolved copper ions (Richardson, 1997).

Like many other fungicides and bactericides, copper is not particularly effective in “curing” an established infection, but rather helps prevent the possibility of infection (Lamichhane et al., 2018; Richardson, 1997). Copper reduces the buildup of inoculum on plant surfaces, decreasing the chance of tissue infection (Lamichhane et al., 2018). Thus, application early in the onset of infection is most effective (Richardson, 1997). Application timing is essential for the success of pathogen management since copper is a contact pesticide and does not easily penetrate plant tissues (La Torre et al., 2018; Lamichhane et al., 2018). It is often most effective during early growth stages or after rainfall when plants may be susceptible to disease (Cabús et al., 2017; Lamichhane et al., 2018).

In field conditions, highly soluble copper compounds like copper sulfate tend to have little protective value, despite their strongly inhibitory effect against microbial spread, because rain and watering remove them from the plant’s surface quickly (Richardson, 1997). Further, deposition into the soil after removal may induce toxicity to the plant and result in significant long-term soil contamination (La Torre et al., 2018; Richardson, 1997). Copper compounds with limited solubility (e.g.,“fixed copper” compounds) carry distinct fungicidal and bactericidal advantages (Lamichhane et al., 2018; Richardson, 1997).

Fixed copper compounds are characterized by their resistance to weathering. These substances supply adequate soluble copper to be toxic to fungi and bacteria without inducing phytotoxicity in the plant host (Richardson, 1997). Given their limited solubility, fixed coppers may form a film on plant tissues that slowly releases copper ions when in contact with water, or with weakly acidic solutions produced by microorganisms or plant secretions (Lamichhane et al., 2018).

The cupric ion’s (Cu^{2+}) mode of action is the nonspecific denaturing of cellular proteins resulting in cell leakage, as well as interference with enzyme active sites (EPA, 2009; Husak, 2015; Lamichhane et al., 2018). The available literature suggests there is still uncertainty regarding some aspects of copper’s mode of action against fungal pathogens (Husak, 2015; Montag et al., 2006). Copper(II) oxide, for example, demonstrates antifungal action despite having almost no ability to solubilize. Montag et al. (2006) compared the efficacy of insoluble copper(II) oxide and barely soluble copper(II) hydroxide suspensions with filtered versions in the treatment of apple scab (*Venturia inaequalis*) in a laboratory setting. The researchers found that filtered suspensions had no inhibitory effect against spore germination. In direct contact with the unfiltered suspensions, however, complete inhibition of spore germination was observed, indicating that free copper ions do not exist in effective amounts in the filtrate.

Solid to solid contact of copper compounds with fungal spore membranes appears to affect the release of copper ions, leading to fungicidal activity (Montag et al., 2006). Montag et al. (2006) propose that secretions (or exudates) from the fungal spore itself assist the solubilization of copper ions, allowing for transport into the cell. Increasing the chance of solid to solid contact by reducing particle size of insoluble copper compounds may help to reduce the amount of material needed to be effective (Montag et al., 2006).

Copper(II) oxide particles generate reactive oxygen species (ROS) on their surfaces and also strongly adhere to bacterial cell membranes (Tamayo et al., 2016). The permeability of the bacterial cell membrane increases due to those two factors, allowing copper(II) oxide to pass through the cytoplasmic membrane uncontrollably, killing the cell (Tamayo et al., 2016). The biocidal nature of this system is two-fold; ROS' induce deadly oxidative stress on the cell, and copper(II) ions react with phosphorus and sulfur compounds in proteins and DNA, disrupting cellular metabolism (Tamayo et al., 2016). Smaller particles are significantly more effective as bactericides, and research into copper nanoparticles is of particular interest (Tamayo et al., 2016).

Combinations of the Substance:

Several of the copper compounds described in this report may be used as precursors in the production of the other copper compounds covered here (Richardson, 2000). For additional information about starting materials, see *Evaluation Question* #2 below.

Copper pesticides commonly contain inert ingredients in their formulations. Of the 51 products listed as copper sulfate on the OMRI Products List, 44 contain inert ingredients (86.3%) (OMRI, 2022). Of the 80 products listed as coppers, fixed, 79 contain inert ingredients (98.8%) (OMRI, 2022). These inert ingredients serve a wide variety of functions including emulsification, dispersion, defoaming, dilution, pH adjustment, and chelation, and consist of soaps, acids, bases, minerals, thickeners, and other EPA List 4 inert ingredients (OMRI, 2022).

Wetting agents, or "spreaders," are commonly included in copper formulations to increase their coverage on plant surfaces by reducing the surface tension of water droplets (Richardson, 1997). Spreaders also increase the potential for runoff into the soil, which may induce toxicity to the plant, necessitating the use of "sticker" adjuvants as well (Richardson, 1997). Fixed coppers are often less adherent than more soluble copper compounds like copper(II) sulfate, and require the inclusion of stickers, sometimes oils, to help prevent runoff (Richardson, 1997). The ability of the pesticide to adhere to the treated surface is known as the "tenacity" of the substance (Richardson, 1997).

Copper oxychloride products are sometimes mixed with elemental sulfur as a dual-active fungicidal formulation (OMRI, 2022). Copper(II) sulfate may be mixed with calcium hydroxide (lime) to form the Bordeaux mixture, a disease control formulation extensively used from the 1880s to 1930s before being partially replaced with slower-release fixed copper compounds (Richardson, 1997). Lime works to "safen" the copper(II) sulfate, neutralizing some acidity of copper sulfate and slowing the release of copper(II) ions by binding them in a slurry of various copper(II) sulfate basic compounds (Beckerman et al., 2008; Richardson, 2000).

Status

Historic Use:

Copper has been a part of human culture for thousands of years, but it did not have a prominent role in agriculture until the 1880s (Richardson, 1997). Alexis Millardet published work in 1885 showing the effective control of downy mildew in grapes by combining copper sulfate with lime in the form of "Bordeaux mixture"(Ayres, 2004; Richardson, 1997). Use of Bordeaux mix quickly spread from France to elsewhere in Europe and North America to control potato blight (Ayres, 2004; Fagnano et al., 2020; Richardson, 1997). By the mid-1930s, producers began to replace Bordeaux mixture or copper sulfate with low-solubility fixed copper alternatives; the fixed copper pesticides were easier to handle and caused less damage to plant foliage (Richardson, 1997). Burgundy mixture is a combination of copper sulfate and sodium carbonate that was also developed around 1885 (Lewis et al., 2016). Burgundy mixture is now considered largely obsolete (Lewis et al., 2016).

Currently, copper is most commonly used to control grape downy mildew, apple scab, and potato late blight (Coelho et al., 2020). In the United States, copper is currently used most predominantly in orchard crops (including nuts, stone fruit, and citrus), rice (to control invertebrates and algae), and tomatoes (EPA, 2009). The 2009 EPA RED report for coppers includes estimated usage amounts for copper products in conventional agriculture. The Screening Level Usage Analysis (SLUA) estimates the annual usage amount of copper hydroxide and copper sulfate pentahydrate, and is included at Table 5 for a selection of the highest-input crops in the US (EPA, 2009). These values include all pesticidal uses, some of which may not be for plant disease control.

Table 5: Estimated annual copper usage in the U.S. for a selection of crops. Adapted from US EPA, 2009.

Crop	Copper Hydroxide (lbs)	Copper Sulfate Pentahydrate (lbs)
Almonds	600,000	100,000
Apples	100,000	60,000
Avocados	100,000	9,000
Cherries	100,000	50,000
Grapefruit	700,000	100,000
Grapes	400,000	100,000
Onions	100,000	10,000
Oranges	1,800,000	900,000
Peaches	200,000	100,000
Peppers	200,000	30,000
Potatoes	90,000	30,000
Prunes & Plums	100,000	30,000
Rice	10,000	300,000
Tomatoes	800,000	40,000
Walnuts	1,400,000	200,000

The report also included estimates from the Copper Sulfate Task Force (CSTF) that 9-11 million pounds of elemental copper is applied in the form of copper sulfate pentahydrate solely for control of algae and weeds each year (EPA, 2009).[6] Additionally, the Applied Biochemists Company estimated another 300,000 pounds of elemental copper is applied annually in the form of complexed copper compounds for algae and weed control (EPA, 2009).

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[6] One pound is equal to approximately 0.454 kilograms (kg).

Some countries have established maximum annual copper limits (see *International* section, below). However, the United States has not defined a universal maximum threshold. The US EPA's 2009 report includes an appendix of "Copper Refined Actual Use Rates for Crops," which includes recommended maximum annual rates for individual crops (US EPA, 2009). We have adapted that information in Appendix B, at the end of this report.

Between 2012 and 2017, producers in California increased the number of acres treated with copper pesticides (CDPR, 2019). The number of acres in California treated with copper pesticides increased for the production of carrots, oranges, peaches and nectarines, tomatoes, table and wine grapes, and walnuts. Overall the amount of acres treated with copper in California increased from just over 2 million acres in 2012 to 2.5 million acres in 2017 (CDPR, 2019).

A high-end estimate of the amount of copper used in all agricultural production (not just for plant disease control) for the years 1992 to 2017 for the continental US (see Table 6) shows that producers used more than 5 million kilograms of copper-based pesticides annually (Wieben, 2020).[7] These data include the major crop or crop groups of corn, soybean, wheat, cotton, vegetables, rice, orchards, alfalfa, pastures, and other crops. There was an almost 20% decrease in the amount of copper-based products used between 1992 and 2017.

Table 6: High-estimate of copper usage in the US from 1992 to 2017 from Wieban, 2019.

Substance	Year	Amount Used (kg)	Year	Amount Used (kg)
Copper	1992	31052.7	2017	531314.4
Copper Hydroxide	1992	2857932.9	2017	2204157.9
Copper Octanoate	2005*	66	2017	12446.6
Copper Oxychloride	1992	11121.8	2017	290000.6
Copper Oxychloride S	1992	134195.4	2017	16516.7
Copper Sulfate	1992	3003942.5	2017	1270871.4
Copper Sulfate Tribasic	1992	307862.4	2017	638114.2
Cuprous Oxide	1992	86492.1	2017	193851.6
	Total:	**6,432,665.8**		**5,157,273.4**

*2005 is the first year more than 1 kg of use reported for copper octanoate.

Organic production is heavily reliant on copper as a pesticide, especially as a fungicide (Coelho et al., 2020; La Torre et al., 2019). Specifically, the organic production of potatoes, tomatoes, apples, citrus, stone fruit, and nuts rely on these materials (Tamm et al., 2022; van Bruggen & Finckh, 2016). Producing crops organically does not necessarily reduce pesticide use, especially in copper-reliant crops. For example, recent work by Larsen and others (2021) described the pesticide use for significant crops in one county in California, and they found wide disparity between the amount of pesticides applied to each crop. Producers applied nearly 25% more pesticide products to organically produced grapes than to conventional grapes (Larsen et al., 2021). Grapes are a crop that typically relies on high copper usage (La Torre et al., 2019). In contrast, producers applied 92% less pesticidal material to organically produced carrots than to conventional carrots (Larsen et al., 2021).

Organic Foods Production Act, USDA Final Rule:

Copper is included in the Organic Foods Production Act of 1990 (OFPA) at 7 U.S.C. 6517(c)(B)(i) and is exempt from prohibition when used as an active synthetic ingredient in production. Because copper is included in OFPA, it also included in the USDA organic regulations on the National List in 7 CFR 205.601(a)(3), 205.601(e)(4), 205.601(i)(2)-(3), and 205.603(b)(1).

The NOSB recommended adding copper sulfate to the National List in 1995 as an allowed active ingredient for plant disease control (NOSB, 1995a). Copper sulfate is currently listed in § 205.601(i) with an annotation requiring the substance to be used in a manner that minimizes accumulation of copper in the soil. In 2003, the NOP responded to NOSB recommendations and added copper sulfate to the National List as an algicide in aquatic rice systems in § 205.601(a), and as an insecticide in § 205.601(e) to control tadpole

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[7] One kilogram (kg) is equal to approximately 2.204 pounds.

shrimp in aquatic rice production (NOSB, 2001). Both instances allow one application during any 24-month period, and require the producer and certifying agent to agree on application levels that do not increase the soil test values for copper.

The NOSB recommended adding fixed coppers substances to the National List in 1995 as allowed synthetic plant disease control materials (USDA, 1995a). Fixed copper compounds are currently listed in § 205.601(i) for plant disease control with a requirement that the substances be used in a manner that minimizes accumulation in the soil, and that the substances shall not be used as herbicides.

Prior to using either copper sulfate or fixed copper substances as pesticides, § 205.206(e) requires producers to use the preventive agricultural practices described in § 205.206(a)–(d). However, copper products need to be applied early in the disease cycle, because they do not cure existing infections (see *Action of the substance*, above). Therefore, the need for using these materials may be based on a past history of infection or some other predictive method.

Based on correspondence with organic certifiers and inspectors, the onus for determining whether copper is used in a manner that minimizes soil accumulation is often left to inspectors (OMRI, personal communication, May 16, 2022). One certifier and, separately, an inspector, indicated that it was difficult to evaluate whether accumulation was occurring in excess of what the rule allows. For example, visual indicators of copper excess (reduced growth, reduced yield, chlorosis) also resembles copper deficiency (Adrees et al., 2015). According to § 205.103(b)(3), operators are only required to keep records for 5 years; therefore inspectors may not be able to look at soil tests over a long enough period of time to assess accumulation. Some certifiers evaluate compliance based on whether application rates are less than or equal to label rates. One certifier also noted that multiple copper products are sometimes used on operations. There is a concern over this, ostensibly because using multiple products, even if at label rates, might cause accumulation beyond what is allowed. Furthermore, because copper dissipates from soil so slowly, simply following label rates does not guarantee that accumulation will not occur. Guidance does not exist on how to evaluate whether a producer is using copper products according to the requirements of the National List annotations. It is therefore challenging for some certifiers and inspectors to know what actionable limits or other benchmarks should be used (OMRI, personal communication, May 16, 2022).

International

A survey of international regulations and guidelines for organic production around the world shows that copper sulfate is permitted as a disease control substance in all regimes (see Table 7). There is more variety in the allowance of fixed copper substances as a pesticide—most regulations permit the use of copper oxide, copper hydroxide, copper oxychloride, and copper octanoate. All guidelines and regulations require that producers attempt to control diseases and other pests with preventive and cultural practices before using copper substances.

Table 7: Allowance of Copper Substances by Global Organic Production Regulations & Guidelines

Substance (CAS No.)	Allowed by NOP	Allowed by CAN/CGSB	Allowed by Codex Alimentarius	Allowed by EEC	Allowed by JAS	Allowed by IFOAM
Copper sulfate (7758-98-7)	X	X	X	X	X	X
Copper hydroxide (20427-59-2)	X	X	X	X		X
Copper oxide/ Cuprous oxide (1317-39-1)	X	X	X	X		
Copper oxychloride (1332-65-6)	X	X	X	X		X
Bourdeaux mix		X	X		X	

Substance (CAS No.)	Allowed by NOP	Allowed by CAN/CGSB	Allowed by Codex Alimentarius	Allowed by EEC	Allowed by JAS	Allowed by IFOAM
(Copper sulfate, lime, water)						
Burgundy mix (Copper sulfate, sodium carbonate)			X			
Copper octanoate (20543-04-8)	X	X		X		X

Canadian General Standards Board Permitted Substances List

The Canadian General Standards Board includes copper sulphate, copper hydroxide, copper octanoate, Bordeaux mix, copper oxychloride, and copper oxide at CAN/CGSB-32.11-2020 Table 4.2, *Substances for crop production* Column 2, as production aids. The entry includes the annotation that the substances may be used as wood preservatives or "for controlling pests, including diseases." Additionally, producers must use the copper substances in a way that prevents excessive copper accumulation in the soil, and the regulations state that copper build-up in the soil shall prohibit future use of the substances. The annotation also states that "visible residue of copper products on harvested crops is prohibited."

The *Organic Production Systems standards* (CAN/CGSB-32.310-2020) state that producers shall focus on organic management practices in order to control crop pests. Cultural, mechanical, and physical pest controls must precede the use of substances included on the *Permitted Substances List* (CAN/CGSB-32.311-2020), as production aids.

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

The Codex Alimentarius guidelines include copper substances on Annex 2, *Permitted Substances for the Production of Organic Foods* in Table 2, *Substances for Plant Pest and Disease Control*. Copper is allowed in the forms of copper hydroxide, copper oxychloride, (tribasic) copper sulphate, cuprous oxide, Bordeaux mixture, and Burgundy mixture. The guidelines indicate that the use and application rates of copper must be recognized by a certification body or authority. Additionally, the producer must use the copper substance in a way that minimizes accumulation in the soil.

The Codex guidelines require that producers utilize appropriate cultural, mechanical, and physical methods to control pests before using products or substances listed in Annex 2.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008

The European Economic Community regulations (EC No. 889/2008) include copper substances in Annex II *Pesticides – plant protection products referred to in Article 5(1),* on Table 6, *Other substances from traditional use in organic farming*. The copper products included are copper hydroxide, copper oxychloride, (tribasic) copper sulphate, cuprous oxide, and copper octanoate. The regulations state that the copper substances must be used as fungicidal materials, up to 6 kg of copper per hectare per year, although member states are permitted to calculate on a rolling average over 5 years.

Article 5 of Chapter 1 of the regulations specifies that producers may only use products listed in Annex II if preventive measured are inadequate to provide protection. Operators and producers must keep records documenting the need for the use of copper products.

Japan Agricultural Standard (JAS) for Organic Production

The Japanese Agricultural Standard for Organic Plants (Notification No. 1605, 2005), last revised in March 2017, includes several forms of copper on Attached Table 2[8] for use as plant pest and disease control. The

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[8] This is the name of the table. It is not attached to this report.

allowed substances are sulfur/copper wettable powder, sodium hydrogen carbonate/copper wettable powder, copper wettable powder, copper powdered agent, copper sulfate, and biopesticide formulation/copper wettable powder. The materials listed in the JAS standards are predominantly descriptions of the physical state of the compounds and are not directly relatable to the substances specified by other organic standards in Table 7 (above). The only limitations included on Attached Table 2 are to limit the use of copper sulfate for the preparation of Bordeaux mixture.

Article 4, *The criteria of the production methods for plant products are as follows,* specifies that producers shall use cultural, physical, and mechanical methods to control pests and diseases before using substances in Attached Table 2, including copper substances.

International Federation of Organic Agriculture Movements (IFOAM – Organics International)
The IFOAM Standard for Organic Production and Processing includes several copper products in Appendix 3 *Crop protectants and growth regulators*. Copper salts (copper sulfate, copper hydroxide, copper oxychloride, copper octanoate) are included for use as crop protectants. The standard limits producers to a maximum use rate of 6 kg/ha of copper per year on a rolling, average basis.

Section 4.5 of the IFOAM Standard includes requirements for the management of pests, weeds, and diseases. Producers must use biological, cultural, and mechanical methods to control pests and disease before they are permitted to use substances on Appendix 3, including copper salts.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?
(A) Copper sulfate and fixed copper substances are "copper and sulfur compounds."

(B) Copper(II) hydroxide (CAS No. 20427-59-2) is included on the 2004 EPA List 4B. No other form of fixed copper substance nor copper sulfate is included on either 2004 EPA List 4A or 4B.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).
The processes of mining, extraction, beneficiation, and refining of copper metal are described in *Source or Origin of the Substance,* above. An enormous variety of copper compounds used throughout countless industries are ultimately derived from the extraction of copper from mineral resources. Below, we describe prevalent processes for manufacturing those compounds relevant to crop protection functions. See also Evaluation Question #3 below and Table 8 for a summary of reactants and processes used to make these substances. This section relies heavily on Richardson (2000).

Copper(II) sulfate
Most commonly, copper(II) sulfates are produced through direct addition of sulfuric acid to blister copper or copper shot, with heat, in the following chemical reaction (Richardson, 2000).

$$Cu + H_2SO_4 + \frac{1}{2}O_2 \rightarrow CuSO_4(aq) + H_2O$$

Copper metal + sulfuric acid + oxygen → dissolved copper(II) sulfate + water

The resulting concentrated copper sulfate solution can then be dried into copper(II) sulfate pentahydrate, or used in a liquid state for the production of other copper compounds (Richardson, 2000).

Liquid copper(II) ammonia complex solutions may also be used as a starting material for copper(II) sulfate production (Richardson, 2000). Organic (carbon-bearing) extractants with an affinity for copper, often acidic unsaturated carbon chains, are added to the solution to selectively "capture" copper ions, liberating ammonia gas (NH_3) and ammonium ions (NH_4^+). The copper rich organic phase is then reacted with sulfuric acid to produce copper sulfate and another organic acid. Cooling and evaporation result in copper(II) sulfate pentahydrate (Richardson, 2000).

Some copper(II) sulfate pentahydrate is recovered as a by-product of the electrolysis process (electrowinning) described above (Richardson, 2000). When solutions with significant impurities preclude the production of pure cathode copper in an electrolysis system, they may be crystallized directly into agricultural grade copper(II) sulfate pentahydrate (Richardson, 2000).

Since all these production methods involve the refining of natural, mineral copper sources using oxidation/reduction reactions or leaching with strong mineral acids, followed by ionic displacement reactions, they represent synthetic processes.

Different hydrates of copper(II) sulfate may be prepared by carefully controlling temperature during drying of copper(II) sulfate pentahydrate, but these compounds are comparatively unstable and must be stored and transported under controlled atmospheric conditions (Richardson, 2000).

Copper(II) sulfate basic

Copper(II) sulfate basic (tribasic copper sulfate) is most often produced by the addition of sodium carbonate to hot solutions of copper(II) sulfate in the following displacement reaction, precipitating copper(II) sulfate basic (Richardson, 2000).

$$4CuSO_4 + 3Na_2CO_3 + H_2O \rightarrow CuSO_4 \cdot 3Cu(OH)_2 + 3Na_2SO_4 + 3CO_2$$

copper(II) sulfate + sodium carbonate + water → copper(II) sulfate basic + sodium sulfate + carbon dioxide

In agricultural preparations, where small particle sizes are important for liquid suspensions, careful temperature control during precipitation must be achieved to avoid the formation of hydrates and inclusion of sulfates in the final crystalline product (Richardson, 2000).

Several other manufacturing processes may be used to produce more economically minor sources of copper(II) sulfate basic, including (Richardson, 2000):

- Copper(I) oxide treated with sulfuric acid and water, aerated with oxygen
- Copper(I) oxide treated with copper(II) sulfate and water, aerated with oxygen
- Drying of concentrated liquids of copper(II) sulfate mixed with ammonium sulfate
- Neutralization of copper(II) sulfate with ammonia gas
- Reaction of copper(II) sulfate with sodium phosphate and sodium hydroxide
- Aeration of copper(II) sulfate solution in contact with metallic copper

Bordeaux mixture and Burgundy mixture are a slurry of various basic copper sulfate compounds. They have been used extensively as fungicides in the past 150 years, and are produced by mixing copper(II) sulfate pentahydrate with lime (calcium hydroxide) or with soda ash (sodium carbonate), respectively (Richardson, 2000).

All of the described manufacturing processes for *copper(II) sulfate basic* rely on chemical reactions, resulting in synthetic products. The naturally occurring tribasic copper(II) sulfate mineral brochantite occurs in hundreds of locations globally (Merlino et al., 2003). While it may be a component of some copper ores (as an oxidation product of copper sulfides), a review of the literature does not support direct extraction of natural tribasic copper sulfate.

Copper(II) hydroxide

One method for producing copper(II) hydroxide involves the addition of a strong alkali, typically sodium hydroxide, to copper ammonia complex in an oxygen atmosphere, resulting in a high purity material (Lossin & Westhoff, 1997; Richardson, 2000).

Another process involves a two-step chemical reaction beginning with copper(II) sulfate (Richardson, 2000).

$$3CuSO_4 + 2Na_3PO_4 \rightarrow Cu_3(PO_4)_2 + 3Na_2SO_4$$

copper(II) sulfate + sodium phosphate → copper(II) phosphate + sodium sulfate

$$Cu_3(PO_4)_2 + 6NaOH \rightarrow 3Cu(OH)_2 + 2Na_3PO_4$$

copper(II) phosphate + sodium hydroxide → copper(II) hydroxide + sodium phosphate

The second process is more often used for agricultural preparations due to a lesser purity but greater stability (Richardson, 2000).

As displacement reactions, these are synthetic manufacturing processes.

Copper(II) oxide

The most common method for producing copper(II) oxide is a multi-stage reaction that starts with metallic copper (Richardson, 2000). Copper metal is oxidized in the presence of ammonia gas into an ammonium copper carbonate complex, which is filtered and subjected to steam, releasing ammonia gas and carbon dioxide and resulting in black copper(II) oxide (Richardson, 2000).

Other methods include (Richardson, 2000):

- Heating copper metal in air at temperatures between 300 and 800 °C
- Spraying molten copper into an oxygen atmosphere
- Igniting copper(II) nitrate
- Heating copper(II) carbonate hydroxide above 250 °C
- Heating copper(II) hydroxide above 100 °C

Cuprous oxide

Copper(I) oxide is produced in many ways, but easily oxidizes to copper(II) oxide, so production and storage parameters must be carefully controlled (Richardson, 2000).

- Copper metal powder, is heated to at least 1030 °C in the presence of air, where it oxidizes to copper(I) oxide. It must be cooled away from oxygen to prevent further oxidation.
- Copper(II) oxide can be heated above 750 °C alongside carbon without oxygen. This form must be coated with another substance like pine oil or isophthalic acid to prevent oxidation back to copper(II) oxide.
- Copper(II) oxide can be blended with metallic copper powder and heated to 800-900 °C without oxygen.
- At pressures approximately 6 times atmospheric pressure, copper metal can be oxidized to copper(I) oxide in air alongside small amounts of mineral acids.
- Copper ammonium sulfate complex or copper ammonium carbonate complex yields copper(I) oxide when pH is adjusted to 3-5.
- Saturated solutions of copper ammonium carbonate can be mixed above a bed of copper metal to form copper(I) oxide on the surface of the metal.
- Copper salts may be leached with organic chelating agents or ammonia gas in pressurized vessels containing carbon monoxide or hydrogen. With addition of sodium hydroxide and an alkaline metal iodide catalyst, copper(I) oxide results.

- Bubbling sulfur dioxide through a boiling suspension of copper(II) sulfate basic results in copper(I) oxide.
- Adding sodium sulfite to a suspension of copper(II) sulfate basic and copper(II) sulfate, adjusting the pH to 3.5-5 and boiling results in copper(I) oxide alongside sulfurous acid.
- A solution of copper(I) chloride and sodium chloride may be neutralized with sodium hydroxide under pressure, resulting in copper(I) oxide.

Copper(II) oxychloride

The most common method for producing copper(II) oxychloride starts with a copper(I) chloride precursor. First, copper(I) chloride is prepared using one of two methods (Richardson, 2000).

- Copper metal is heated to red hot and chlorine gas is introduced. This may involve solid-state spontaneous chemical reaction (requiring a cooling apparatus since the reaction expels heat), or melting into a liquid, which is then cast and crushed.
- Alternatively, copper(II) chloride may be reduced in a chemical reaction. First copper(II) chloride is manufactured by reacting copper(II) oxide, copper(II) carbonate hydroxide, or copper(II) hydroxide with hydrochloric acid resulting in copper(II) chloride. The liquid solution is crystallized. Second, copper(II) chloride is reduced using sodium sulfite (Na_2SO_3), metallic copper, sulfurous acid (H_2SO_3), hydroxylamine (H_3NO), hydrazine (N_2H_4), phosphorous acid (H_3PO_3), or zinc.

Following the initial preparation of copper(I) chloride, copper(II) oxychloride may be prepared by oxidation in air and water, precipitating copper(II) oxychloride.

Copper(II) oxychloride sulfate

Copper(II) oxychloride sulfate (sometimes known as COCS) is the mixture of two distinct active ingredients covered elsewhere, copper(II) oxychloride and copper(II) sulfate basic (EPA, n.d.).

Copper(II) carbonate hydroxide

Copper(II) carbonate is produced by the reaction of a dissolved copper(II) salt (often copper(II) sulfate) with concentrated solutions of either sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$), depending on the desired density and surface area of the final product (Richardson, 2000). Copper(II) ammonium carbonate solution may also be boiled to drive off ammonia and carbon dioxide, resulting in copper(II) carbonate hydroxide (Richardson, 2000).

Copper(II) ammonia complex

As copper(II) ion dissolves in solution, a complex with water molecules forms in which the copper is surrounded by 6 coordinated water ligands (Hathaway & Tomlinson, 1970). Essentially, copper(II) ammonia complex can be prepared by adding ammonia to any dissolved copper(II) solution, thus replacing the water ligands with ammonia (Hathaway & Tomlinson, 1970). Several salts may be used as a copper source including copper chlorides, copper bromides, copper cyanide, copper fluorides, copper iodides, copper sulfates, and copper hydroxides (Hathaway & Tomlinson, 1970; Richardson, 2000).

Copper(II) ethylenediamine complex

The concept behind copper(II) ethylenediamine complex is the same as that for copper(II) ammonia complex, except that copper(II) ions in solution are complexed with ethylenediamine ($C_2H_8N_2$), one of the most commonly used complexing agents in the field of chemistry (Sharma et al., 2019). We were unable to locate specific manufacturing processes for commercial copper(II) ethylenediamine complex, but a review of the available literature indicates that scientific researchers may use copper(II) acetate and copper(II) nitrate in laboratory preparations (Sharma et al., 2019; Sridaeng et al., 2015). It seems reasonable that other soluble copper salts would be appropriate as well.

Copper salts of fatty and rosin acids

Also known as copper soaps, copper salts of fatty acids are prepared by reaction of alkaline copper(II) hydroxide or copper(II) carbonate hydroxide with a fatty acid, followed by dilution with an organic solvent

(Richardson, 2000). They may also be directly prepared by mixing copper(II) sulfate solution with a sodium salt of fatty acid (sodium soap) (Richardson, 2000).

Like fatty acids, rosin acids contain a carboxylic acid group that participates in chemical bonding to form salts (Silvestre & Gandini, 2008). Rosin may be tapped from living pine trees, recovered as a by-product of Kraft pulping of pine for manufacture of wood pulp (sometimes known as tall oil rosin), or extracted with solvents from harvested wood (Silvestre & Gandini, 2008). Though not true "soaps" derived from saponification of fats with alkali, rosin acid salts have been used for the same purposes as soaps (Silvestre & Gandini, 2008).

Copper(II) octanoate

Copper(II) octanoate, one of the copper salts of fatty acids, is prepared by the reaction of a soluble copper compound with a naturally derived fatty acid source such as palm kernel oil (EPA, 1997b; Stamatopoulou et al., 2020).

<u>Evaluation Question #3:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Hundreds of naturally occurring copper minerals have been identified, but few are important as ore resources. Examples of important ore minerals include chalcopyrite (copper iron sulfide, $CuFeS_2$), bornite (copper iron sulfide, Cu_5FeS_4), chalcocite (copper sulfide, Cu_2S) and covellite (copper sulfide, CuS) (Lossin, 2001). None of these compounds are covered in this report as commonly used agricultural pesticides. Naturally occurring azurite (copper carbonate hydroxide), chrysocolla (copper silicate), and turquoise (hydrated copper aluminum phosphate) are primarily collected as rare semi-precious gemstones (Lossin, 2001). These copper minerals are not agriculturally relevant or economically feasible to extract for direct use as pesticides.

The commonly used fungicides and bactericides described in this report are manufactured with chemical processes. The two predominant methods for concentrating copper ore materials (pyrometallurgical and hydrometallurgical) involve breaking chemical bonds (see *Source or Origin of the Substance* above). Since the majority of copper ores are sulfides or oxides, bonds between copper and sulfur, or copper and oxygen are broken in order to isolate the copper. In pyrometallurgical processes, copper metal is separated from sulfur atoms through an oxidation reaction, producing sulfur oxide by-products. In hydrometallurgical processes, sulfuric acid is used to facilitate mobilizing copper ions from the crystal lattice of ore minerals, and then the ions are chemically reduced to metal through electrolysis reactions.

Most commercially important copper fungicides are made directly from refined copper metal, utilizing chemical reactions to create new ionic compounds. A small percentage of total extracted copper goes toward fungicide production (ATSDR, 2004).

Using Guidance NOP 5033-1: *Decision Tree for Classification of Materials as Synthetic or Nonsynthetic* (NOP, 2016), all of the relevant copper pesticide compounds are synthetic materials. Copper is extracted from a natural source, copper ore minerals, but each compound has undergone a chemical change. Table 8 summarizes the chemical reactions commonly used to manufacture copper pesticides.

Table 8: Predominately used manufacturing processes of copper pesticide compounds and chemical changes associated with them

Compound	Chemical Reaction Between	Chemical Reaction Types
copper(II) sulfate	copper metal and sulfuric acid	oxidation/reduction, electrolysis, ionic displacement
copper(II) sulfate basic	copper sulfate and sodium carbonate	oxidation/reduction, electrolysis, ionic displacement
copper(II) hydroxide	copper ammonia complex and sodium hydroxide or copper sulfate, sodium phosphate, and sodium hydroxide	oxidation/reduction, complexation, electrolysis, ionic displacement

Compound	Chemical Reaction Between	Chemical Reaction Types
copper(II) oxide	copper metal, ammonia, and oxygen	oxidation/reduction, complexation, decomposition
cuprous oxide	copper metal and oxygen	oxidation/reduction
copper(II) oxychloride	copper chloride and oxygen	oxidation/reduction, ionic displacement
copper(II) carbonate hydroxide	copper salts, water and sodium carbonate	oxidation/reduction, ionic displacement, hydration
copper(II) ammonia complex	copper salts and dissolved ammonium	oxidation/reduction, complexation
copper(II) ethylenediamine complex	copper salts and ethylenediamine	oxidation/reduction, complexation
copper salts of fatty and rosin acids	alkaline copper salts and fatty acids	oxidation/reduction, saponification, ionic displacement
copper(II) octanoate	alkaline copper salts and fatty acids	oxidation/reduction, saponification, ionic displacement

Source: (Richardson, 2000)

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

In this section, we consider the fate of copper ions as the primary focus, and separately, counter ions and other molecules that are a part of copper products as the minor focus. In many cases, detailed environmental fate data were difficult to find for specific copper products. Throughout the following evaluation questions, we attempted to locate studies specific to copper used on farms or land in the United States; however, in many cases the only relevant peer-reviewed research was from Europe.

According to older literature, the largest inputs of copper to soil are waste products from copper mines and mills (Prasad, 2008). Other sources include (Prasad, 2008):

- mining and smelting activities
- industrial emissions and effluents
- traffic
- fly ash
- dumped waste materials
- contaminated dust and rainfall
- sewage and sludge from wastewater
- pig slurry
- composted refuse
- agricultural fertilizers and pesticides

Agricultural applications of copper products are persistent. Unlike other pesticides that decompose into simple substances like carbon dioxide (CO_2) and water, copper is an element that does not decompose outside of nuclear reactions. It binds to both organic and inorganic matter in the soil, and is not destroyed by either sunlight or soil organisms. In order to be effective, copper products must release the free copper ion (see *Action of the Substance*). As a generalization, copper products all break down into free copper ions and counter ions or other formerly attached molecules (see *Properties of the Substance*).

Some copper products are more stable than others, and thus remain in the soil (as applied) longer than others. For example, unlike many other copper products, copper(II) sulfate is highly soluble in water (see *Appendix of Chemical and Physical Properties*). It dissolves readily into copper and sulfate ions in the water/soil matrix at low to neutral pH. In contrast, copper(II) oxide is an insoluble mineral that requires reactions with acids or ammonia in order to dissolve (Richardson, 2000).

Copper applied to plants and soils can move into the hydrosphere (waterways) (Prasad, 2008). The primary cause for copper movement into the hydrosphere is soil disturbance, followed by agricultural applications (see Table 9 below) (Prasad, 2008). Aquatic organisms can be particularly sensitive to copper (see *Evaluation Question #5* and *#8*).

Table 9: Causes of copper release into the hydrosphere. Based on data from Perwak et al., 1980.

Cause of copper release	Percent
soil disturbance	68
agricultural application of copper	13
electroplating facilities, mines, other	9
publically owned water treatment works	8
urban runoff	2

Copper ions

Soil is a matrix of inorganic substances, organic (carbon-containing) materials, water, and air. Metals such as copper associate with different parts of this matrix. Copper may be (EPA, 1992; Alloway, 2013):

- dissolved in the water-containing (aqueous) fraction
- exchanged with other ions associated with inorganic substances (such as sodium or calcium)
- adsorbed onto the surface of inorganic substances
- bonded to soluble and insoluble organic matter
- found as solid precipitates
- incorporated in the structure of minerals

Copper ions (Cu^{2+}) preferentially bind to substances in the following order: manganese oxides[9] > organic matter > iron oxides > clay minerals (Alloway, 2013). The probability of copper binding to any given substance is influenced by pH, the availability of oxygen (redox potential), and the presence of competing ions (Alloway, 2013).

Soil organic matter (SOM)[10] and dissolved organic matter (DOM)[11] strongly influence the behavior of copper in the soil (Ponizovsky et al., 2006). Copper ions adsorb[12] to both SOM and DOM, and so free copper ions are removed from the aqueous portion of the soil (see Figure 1). An older study estimated that about 36% of copper overall is associated with SOM (Prasad, 2008). Copper itself can cause a decrease in soil DOM content, because it causes these materials to clump (coagulate) and precipitate out of solution. It can also displace other ions, like hydrogen, calcium, magnesium, and sodium that would otherwise be bound by SOM and DOM. Copper ions have an affinity for specific chemical functional groups within the soil, notably phenolic and carboxylic groups (Ponizovsky et al., 2006).

In water, copper can complex with DOM and other substances over the course of days (Stevens et al., 2014). Researchers in Australia applied copper sulfate (3 to 12 kg/hectare) to three separate soil types, which they then covered with water. The amount of copper dissolved in the water decreased over time (varying somewhat by soil type), practically disappearing after seven days, and falling below the detection limit[13] after 20 days (Stevens et al., 2014). However, in an assay using *Pseudomonas fluorescens,* Brandt et al. (2008) found that copper-DOM complexes were still bioavailable forms of copper.

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[9] Manganese oxides are ubiquitous in the natural environment, including within soils (Hullebusch et al., 2020).

[10] Soil organic matter consists of the organic (carbon containing) fraction of the soil, but does not include undecayed plant and animal matter (Sylvia, 2005).

[11] Dissolved organic matter consists of organic material that passes through a filter with 0.5 micrometer pores (Krauss & Nies, 2015).

[12] Adsorption is "the process by which atoms, molecules, or ions are taken up and retained on the surfaces of solids by chemical or physical binding" (Sylvia, 2005)

[13] The detection limit in this study was 0.02 mg of copper per liter.

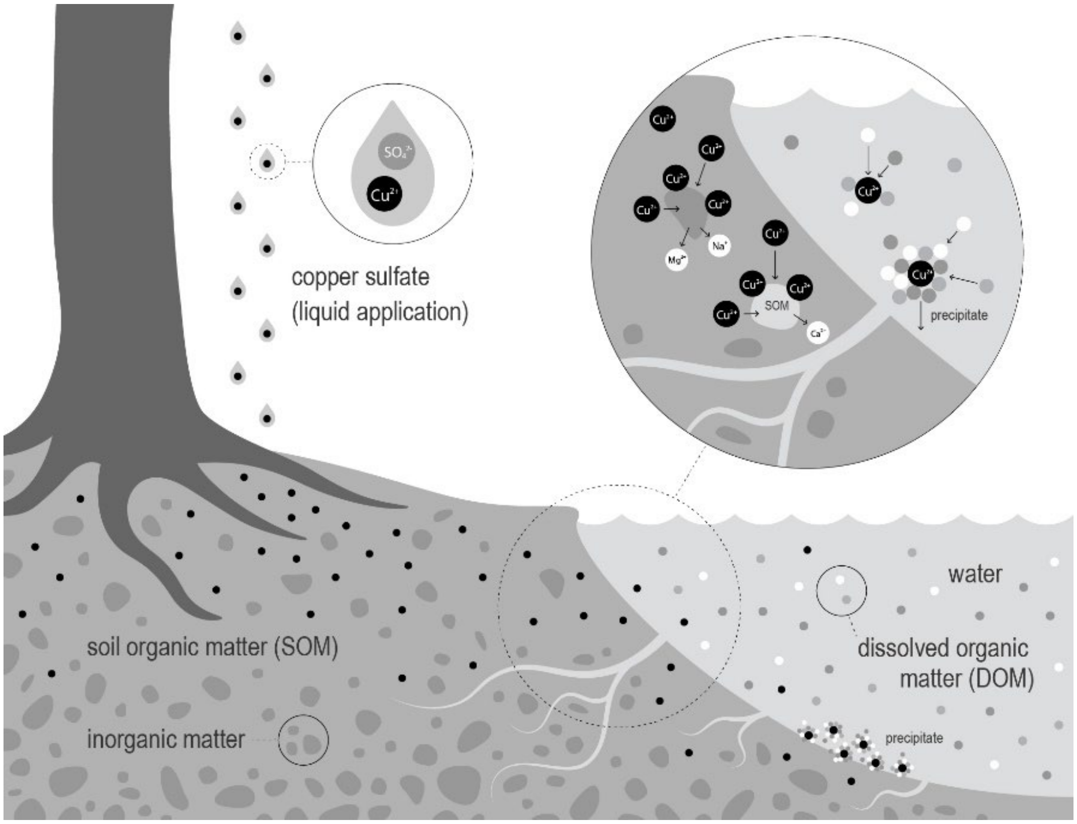

Figure 1: Movement of copper in soil and water.

Meite et al. (2022) found that soil type affected the distribution of copper added in laboratory experiments. Copper had a higher affinity for silty soil (54%) than clay (24%) or sand (23%) in their experiments. They also found that sterile soil had twice as much copper in the aqueous phase of the soil, as compared with nonsterile soil.

Where copper is located in the soil matrix can affect its mobility (Alloway, 2013). Copper can move into both the liquid and solid portions of the soil. Increasing DOM in soils (such as through the application of biosolids or other similar materials) can allow copper to bind to it and become mobile throughout the agroecosystem without actually becoming free copper (Alloway, 2013).

Copper not bound to SOM or DOM exists in a variety of forms, which are pH dependent (Alloway, 2013; Cuppett et al., 2006). At low soil pH (up to around 6), Cu^{2+} ions (free copper) are predominant (Cuppett et al., 2006). When soil pH increases above 7-8, copper becomes less soluble (Alloway, 2013). Between a pH of 6.5–12, copper hydroxide forms, which is nearly insoluble in water (Cuppett et al., 2006). Depending on the counter ions present, salts such as copper hydroxide, copper oxide (the mineral tenorite), and various copper carbonate minerals (e.g., malachite, azurite) form and precipitate out of solution (Alloway, 2013; Cuppett et al., 2006). Copper has a high affinity for phosphorous in soils, and forms insoluble complexes with it (Rippner et al., 2021). In low-oxygen environments, such as saturated soils, copper can be reduced to copper metal or copper(I) sulfides, both insoluble and stable materials (Alloway, 2013).

As free copper is sorbed onto soil organic matter and other substances in the soil matrix, it stabilizes (Alloway, 2013; Buekers et al., 2007). This process is generally referred to as “aging.” Over time, copper can become increasingly difficult to extract from soil, as it diffuses into inaccessible places within organic and inorganic soil particles. For example, Buekers (2007) found that an average of 43% of the copper[14] added to 28 different soils was fixed after 850 days. However, copper aging or fixation is an equilibrium process that is reversible, through processes such as disturbance and flooding (Hoang et al., 2008; Perwak et al., 1980; Sun et al., 2008). Additionally, there may be variability in real-world copper aging. For example, in

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[14] 100 mg Cu per kg of soil (ppm) (Buekers et al., 2007)

vineyards, soils can contain high-levels of bioavailable copper, accumulated over periods greater than 30 years (Girotto et al., 2016).

Changes in soil pH can effect copper aging processes as well. Pedersen (1997) found that decreasing pH increased the amount of copper that desorbed from soil particles. Soils where copper was applied over 100 years ago can still exhibit biological effects (Naveed et al., 2014), so the relevance of aging on ecosystems is complex. These effects are described in *Evaluation Question #8* (below).

On a much smaller scale, the availability of metals is affected by plants and microorganisms (Giller et al., 1998). Plants produce chemical substances that affect the soil directly near the root, or rhizosphere. Some of these substances affect metal bioavailability, and others affect microbial activity. Microorganisms can produce substances that cause extreme acidification, leading to a decrease in pH and an increase in copper solubility (Giller et al., 1998).

An older study conducted by the Soil Conservation Service (beginning in the late 1970's) evaluated agricultural soils and crops from 3,045 sites (Holmgren et al., 1993). While the mean level of copper in agricultural soils at that time was 18 ppm Cu, there was a large range: from 0.6 ppm Cu to a maximum of 495 ppm Cu.[15]

- 10% of agricultural soils had 62 ppm Cu or higher
- 5% of agricultural soils had 94.9 ppm Cu or higher
- 1% of agricultural soils had 216 ppm Cu or higher

Location and soil type are correlated with copper levels. Histosols[16] had the highest average content of 183 ppm Cu, with vertisols[17] (48.5 ppm Cu) and spodosols[18] (48.3 ppm Cu) coming next. Utisols had the lowest average copper content, [19] at 6.2 ppm Cu. Maine, Florida, California, Washington, and Oregon had the highest average copper levels (see Figure 2, below) (Holmgren et al., 1993).

More current surveys of agricultural soils across the U.S. were not located at the time of this report. One more recent USGS database was found, but it did not focus on agricultural soils (though it did include some values). A recent study of copper accumulations on organic and conventional farms in New Jersey is described in *Evaluation Question* #6. It is possible (if not probable) that soil concentrations have changed since this study was conducted, likely with increases in copper. The study by Holmgren et al. (1993) establishes that copper is often elevated in agricultural soils, to the point that in some cases it is likely that soil organisms are affected (see subsequent sections on these levels). In particular, soils high in organic matter are correlated with high copper levels (Holmgren et al., 1993).

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[15] Throughout this report, copper values should be assumed to be for total soil copper levels, unless noted as "bioavailable." However, total and bioavailable copper can be measured through different means, and may not all be exactly equivalent. We have converted mg/kg as reported in literature to ppm. One mg/kg is equal to 1 ppm.

[16] Histosols are soils high in organic matter (20-30%) and are greater than 40 cm thick. Often referred to as "peat" or "muck" soils (University of Idaho, 2022a).

[17] Vertisols are clay-rich soils, at least 30% to a depth of 50 cm (University of Idaho, 2022d)

[18] Spodosols are acid soils with accumulations of humus, complexed with iron and aluminum. These soils are typical of coniferous forests in cool, moist climates (University of Idaho, 2022b).

[19] Utisols are highly leached, acid soils, with layers of clay below the surface. They are common in the southeastern United States (University of Idaho, 2022c).

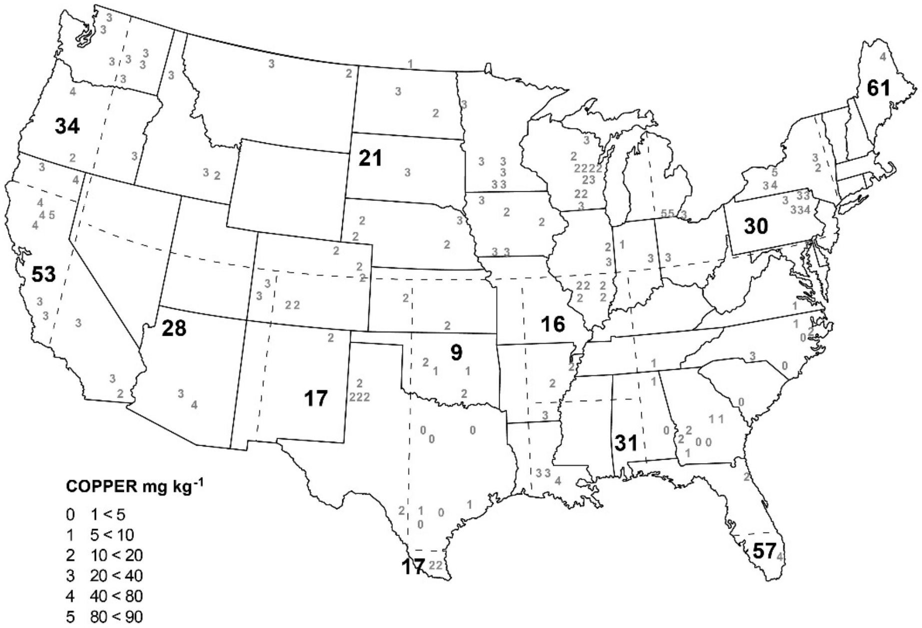

Figure 2: Geographic distribution of copper concentration in U.S. soils around 1978. Areas separated by dotted lines represent statistically distinct areas, based on variance. Bold numbers represent means in mg Cu/ kg soil (equivalent to ppm), whereas small numbers are county averages. Adapted from Holmgren et al., 1993

In the Netherlands, researchers found that copper inputs exceeded outputs in agricultural soils, leading to accumulation (Alloway, 2013). Inputs that the researchers considered included animal manure, fertilizers, use of other organic materials, and deposition from the atmosphere. Outputs included plant uptake and leaching. Copper inputs exceeded uptake and leaching in 97% of the 4,647 plots. The time it takes before negative effects on organisms are observed depends on several factors (Alloway, 2013):

- the amount of copper exceeding balance
- the existing soil copper concentration
- soil chemistry (pH, cation exchange capacity)
- organisms of interest

Sulfate ions

Sulfate ions (SO_4^{2-}) are another product of copper sulfate applications, and are common in well-aerated agricultural soils (Sylvia, 2005). Sulfur is a macronutrient, required for the production of specific amino acids, vitamins, hormones, and other cellular components. Sulfate is converted into other compounds which have many different fates, similar to nitrates entering the nitrogen cycle (Sylvia, 2005).

For example, sulfate ions are used by microorganisms and plants as a nutrient, but can also react with soil constituents, and/or leach into groundwater or other aquatic systems (Sylvia, 2005; Tabatabai, 1987). Like copper, sulfate can also become adsorbed to soil particles (Sylvia, 2005; Tabatabai, 1987). Sulfate can also form salts of low solubility with other ions in the soil matrix, such as barium, strontium, iron, aluminum, and calcium (Tabatabai, 1987). Microorganisms can reduce sulfate to S_2^-, eventually forming hydrogen sulfide gas (Tabatabai, 1987). Sulfur from sulfate cycles between mineralized/immobilized (bound up in biomass or in mineral forms) and demineralized/mobilized forms, driven largely by the activity of microorganisms (Sylvia, 2005).

Hydroxide ions

Depending on conditions, hydroxide ions (OH^-) released from copper products are likely to (Fasching, 1998):

- become neutralized by hydrogen ions (forming water)
- bond to cations in the soil matrix
- increase the pH of the soil

Oxides

Particle size affects the speed at which copper oxide breaks down (Rippner et al., 2021). In one soil study, researchers applied copper oxide with different particle sizes: from nano-sized particles (16 and 42 nanometers) to bulkier 430 nanometer particles (Rippner et al., 2021).[20] After 70 days, 16 nanometer copper oxide particles were no longer detectable in the soil. These small particles dissolved, and the copper was adsorbed to silicates or complexed with soil organic matter (Rippner et al., 2021). As copper oxide particle size increased, so did the amount of copper oxide found remaining in the soil (Rippner et al., 2021). Studies that specifically describe the fate of copper oxide or oxide ions (O^{2-}) in soil or water were not found. However, it is likely that negatively-charged oxide anions would combine with other more reactive cations (such as zinc), or hydrogen (forming water).

Oxychloride

As with oxides, studies that specifically describe the fate of copper oxychloride in soil or water were not found. However, numerous decomposition reactions have been proposed for copper oxychloride in air (Nixon et al., 2012). These reactions involve high temperatures, and the reaction products are substances such as chlorine gas, water, and oxygen gas (Nixon et al., 2012).

Carbonate ions

Carbonates (CO_3^{2-}) are an important reservoir of carbon in soils (Zamanian et al., 2021). Human-caused acidification with nitrogen fertilizers has led to a dramatic loss of naturally occurring carbonates in soil. Carbonates decompose to carbon dioxide gas and water when exposed to acids. (Zamanian et al., 2021).

Ammonia

Ammonia (NH_3) is a nitrogen-containing gas, and volatilizes above 10°C (Gowariker et al., 2008). Many organisms produce ammonia during normal metabolic activity, but subsequently convert it to other substances (such as urea), because it can be toxic. Bacteria convert ammonia to different forms such as nitrite (NO_2^-) and nitrate (NO_3^-). Nitrate is then assimilated by plants (Gowariker et al., 2008).

Ethylenediamine

Ethylenediamine [$C_2H_4(NH_2)_2$] is produced synthetically in large quantities, but no environmental monitoring data exists for the substance (National Center for Biotechnology Information, 2022b). It is estimated to be slightly mobile in soils, but adsorbs to solids in both soil and water. While experimental data is not available, the Hazardous Substances Data Bank suggests that ethylenediamine biodegradation is the primary decomposition mechanism in soil and water (National Center for Biotechnology Information, 2022b).

Octanoate/fatty and rosin acids

According to Richardson (2000), the common copper soaps include copper(II) napthenate, oleate, and stearate. However, only copper(II) octanoate appears to be used for agricultural purposes (see *Historic Use of the Substance*).

Octanoate is the conjugate base of octanoic acid. It is a medium chain fatty acid that is readily used as an energy source (Sam et al., 2021).[21] The US EPA (1997a) hypothesizes that octanoic acid is degraded by microorganisms.

Application and accumulation rates

Published data summaries on the total application of copper pesticides in the United States are limited. Lamichhane (2018) notes that there is a "serious lack of data on copper used in agriculture." Richardson (1997) provides data on worldwide fungicide copper use, but this data is now over 30 years old. The EPA's 2009 Reregistration Eligibility Decision report (EPA, 2009) includes maximum single and cumulative

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[20] Copper was applied at a concentration of 1000 mg per kg of soil (ppm).

[21] Octanoic acid is composed of a chain of eight carbon atoms. Medium chain fatty acids range from 6-10 carbon atoms long (Sam et al., 2021).

application rates for copper used as a fungicide, bactericide, algaecide, herbicide, leech control, freshwater snail control, anti-foulant and wood preservative. This data is summarized in Appendix B, at the end of this report. According to the EPA, actual use rates are lower than maximum allowable levels, based on input from users (EPA, 2009). However, Lamichhane (2018) indicates that the number of applications per season in some instances (up to 15) is significantly higher than that assumed by EPA (four). Alloway reported that vineyards in France typically receive three to ten applications (2013). Furthermore, because copper doesn't degrade, it generally accumulates in soils.

Citrus, tree nuts, tomato, pepper, grape, berries and peach are crops that receive large copper applications (EPA, 2009). Globally, vineyards and orchards typically receive between 1 and 11 kg Cu/hectare/year (Rippner et al., 2021). In these soils, copper can accumulate at a rate of at least 3–5 mg/kg soil/year (Alloway, 2013; Rippner et al., 2021). In some places however, copper can be applied at up to 80 kg/hectare/year due to repeated applications (Lamichhane et al., 2018).

Background levels of copper in soil typically range from 2—50 ppm, but can be higher or lower in some soils (Alloway, 2013). In France, vineyards with a long history of use can have soil concentrations of approximately 1500 ppm, while those in Brazil can have up to 3215 ppm (Lamichhane et al., 2018). Copper typically accumulates in the top 15cm of soil (Lamichhane et al., 2018). Immediately after application, aerosol drift and runoff from foliar fungicides can elevate copper levels to 30,000 mg Cu/L of soil (or approximately 22,556 ppm Cu[22]) at the soil surface (Rippner et al., 2021).

In Brazil, soils with a history of grapevine cultivation of over 30 years have 30–60 times more bioavailable copper, compared to nearby forest soils (Girotto et al., 2016). These same soils have only 6.5–16 times more total copper than forest soils. This difference between bioavailable copper and total copper is due to a) the relatively low amount of bioavailable copper in forest soils compared to their total copper; and b) the relatively high proportion of bioavailable copper in vineyard soils due to the application of copper fungicides. In other words, even after 30 years, the copper from fungicide sprays remains primarily in bioavailable forms (Girotto et al., 2016).

Over 1.5 million kg of copper were applied as a fungicide or algaecide to just under 1 million hectacres of land in California in 2017, or approximately 1.5 kg per hectacre (Rippner et al., 2021). For comparison, industries released roughly 635 million pounds (288 million kilograms) of copper into the environment on Earth in the year 2000 alone (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). From 2017-2018, copper was the most used fungicidal active ingredient by acreage overall in California (CDPR, 2020). During that same time, copper was the most used fungicide by acre specifically for table grapes, walnuts, and oranges. It was the 5th most used fungicide for almonds (CDPR, 2020).

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Persistence and concentration of copper products is discussed in *Evaluation Question* #4.

An in-depth review of copper ecotoxicology was requested within the scope of this TR . In order to accommodate this, we have limited discussion of toxicity and mode of action to copper itself. Covering all copper products and their breakdown products would be impractical. However, based on the responses in *Evaluation Question #4*, we expect that the materials associated with copper products are short-lived relative to copper itself, decomposing into common organic and inorganic substances. Human health considerations are discussed later in *Evaluation Question #10*, as well as *Focus Question #1*.

While an attempt was made to separate responses in *Evaluation Question #5* and *#8*, these questions are interrelated. Copper products can negatively affect the survival and behavior of many soil organisms.

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[22] Assuming an average bulk soil density of 1.33 grams per cm^3, based on a typical silty loam (NRCS, n.d.). Cited values were 30,000 mg per liter of soil (Rippner et al., 2021).

Descriptions of this, including known incidents and potential for incidents to occur within the agro-ecosystem, are described in *Evaluation Question #8*. Here, we will focus on the mode of action of copper.

Copper is both an essential element, and toxic at excessive concentrations (Alloway, 2013). What constitutes "excessive," is complex and variable, depending on numerous factors including the organisms of interest, soil chemistry, and past history of copper inputs to the soil. While not common, bioavailable copper can be deficient in soils or in intensive farming systems. Copper concentration in healthy plant tissue dry matter ranges from 4—15 ppm. It is often incorporated into proteins as a cofactor (helper molecule), such as in the case of cyctochrome C oxidase[23] (Alloway, 2013).

Excessive levels of bioavailable copper can have toxic effects on plants, invertebrates, and microorganisms (Alloway, 2013). The bioavailability of copper depends on various factors (as described in *Evaluation Question #4*), including soil pH, redox potential, and the organic matter available in soils. These factors have a large impact on the toxicity of copper on soil organisms (see Table 10, below). Copper tends to become less bioavailable as it ages, and thus less toxic. However, this is not a permanent process, and can be reversed. Depending on soil conditions, copper concentrations of 10-200 ppm are expected to be safe for 95% of organisms (Alloway, 2013). See *Evaluation Question #8* for a field study demonstrating the critical concentration of copper for various soil organisms. Agricultural applications of copper fungicides can lead to levels exceeding this range; however this usually takes many years (Alloway, 2013; Eisler, 1998; Lamichhane et al., 2018). Other copper sources (such as manure) contribute to the overall copper balance of soils, and can accelerate total accumulation (see *Focus Question* #2).

Table 10: Concentration of copper in soil corresponding to a 10% inhibition response (EC10) in laboratory experiments. Adapted from Alloway (2013), summarizing European Chemicals Agency (ECHA) data.

Organism	Range (mg Cu/kg soil)	Number of data (tests)
monocotyledon plants	18-537	32; 5 species, 3 families
dicotyledon plants	36-698	35; 4 species, 2 families
arthropods	31-1460	57; 5 species, 3 families
annelida and nemotoda	8.4-728	51; 5 species, 3 families
microorganisms (C transformation)	30-2402	49, 4 processes
microorganisms (N transformation)	31-1270	26; 4 processes
microorganisms (biomass)	150-500	2

Copper ion toxicity in plants

In plants, phytotoxic effects include (Alloway, 2013):

- reduction of root elongation
- abnormal root branching
- root discoloration
- leaf chlorosis
- reduced plant growth

Plants have mechanisms to regulate copper levels in tissues (Alloway, 2013). While copper can build up and is generally better tolerated in roots, plants can regulate how much of this moves to shoot tissues. The onset of copper toxicity in plant roots (critical concentration) varies between approximately 100–400 ppm Cu in dry matter. However, the critical concentration of copper in plant shoots is usually much lower—often between approximately 5–40 ppm Cu in dry matter (Alloway, 2013).

Copper toxicity occurs prior to significant accumulation in plant shoots. In one experiment, black oats were grown in forest and vineyard soils from Brazil, with copper concentrations of 2.2-328.7 ppm bioavailable Cu (Girotto et al., 2016). In the vineyard soils forty days after emergence, copper accumulated in the plant roots, but very little translocated to the shoots. In the forest soil controls with 2–5 ppm bioavailable Cu,

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[23] Cytochrome C oxidase is an important metabolic protein common to all eukaryotic organisms (those with membrane-bound organelles), and many aerobic bacteria (Wikström & Sharma, 2018).

shoots and roots had similar concentrations of copper. Plants showed symptoms of phytotoxicity, including reduced root and shoot size, beginning with the lowest concentrations of copper in vineyard soils (36.8 ppm bioavailable Cu, 51.6 ppm total Cu) (Girotto et al., 2016).

Some plants have the ability to tolerate higher levels of copper, and can be used to remediate soil (Coelho et al., 2020). For example, amaranthus, mustard, and sunflower shoot tissue contains higher copper levels than roots. Biomass harvested from these plants can be used to clean copper and other heavy metal pollution (Coelho et al., 2020).

Free copper ions are highly reactive, and cells have mechanisms to chelate or otherwise bind copper ions to limit their activity (Gupta & Sandalio, 2012). When the concentration of copper ions exceeds what can be detoxified, it reacts with cellular substances like hydrogen peroxide, forming potentially damaging free radicals called "reactive oxygen species" (ROS).[24] Cells have mechanisms to regulate ROS as well as copper, but at a certain point, this challenge exceeds the cell's ability to respond. These unchecked ROS initiate a cascade of events, leading to the damage of cellular components. These components include cell membranes and mitochondrial enzymes in particular, but affect all types of biomolecules. In the case of plants, destruction of lipids causes membranes to become more permeable, leaking ions such as potassium from roots, which led to plant stress. Excess copper can also stop the production of new mitochondria, and affect the proper function of existing ones (Gupta & Sandalio, 2012).

It is likely that excess copper can also displace magnesium from chlorophyll in plants, disrupting photosynthesis (Küpper et al., 1996). This toxic effect has been demonstrated in aquatic plants, with lethal doses from 0.5-20 µmol /L (or approximately 0.08–3.19 mg copper sulfate/L), depending on the plant species (Küpper et al., 1996).

Copper ion toxicity in invertebrates

While it is well established that copper is toxic to invertebrates, literature does not describe a single, uniform mode of action. Copper can cause damage to the gills of some invertebrates, interfere with oxygen transport, cause hypoxia in tissues, and interfere with the production of ATP (Eisler, 1998). Copper can disrupt the function of enzymes that normally protect membranes and other cellular components (Eisler, 1998).

In a study on the red earthworm *Lumbricus rubellus*, Bundy et al. (2008) concluded that sub-lethal concentrations of copper (40 ppm Cu and higher) disrupted the function of mitochondria and lysosomal enzymes. This pattern is similar to that found in plants, where ROS damages the function of enzymes and membranes.

The effects of copper on invertebrates is not always uniform, and may vary depending on the route of exposure (soil/water contact vs. ingestion) (Hook et al., 2014). In a study on copepods, reproductive toxicity occurred when the animals consumed copper-contaminated algal cells, but did not occur when exposed to the same amount of copper dissolved in water. In a study on the amphipod *Melita plumulosa*, Hook et al. found that the exposure route of copper (dissolved in water containing the organism vs. ingested) produced different responses in gene transcription.[25] The researchers concluded that this was due to copper exhibiting different modes of action, depending on exposure route. Exposure via copper dissolved in water caused increases in mRNA related to the cytoskeleton, whereas ingested copper caused decreases in mRNA related to digestive enzymes. Copper itself doesn't necessarily directly cause the change in mRNA, but rather may cause damage that the cell responds to, leading to changes in gene activation in order to maintain homeostasis. They also found that higher concentrations of dissolved

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[24] Reactive oxygen species can include hydroxyl (OH) radicals, which are produced directly by the activity of copper ions on hydrogen peroxide (called the "Fenton reaction"), forming hydroxyl radicals and the hydroxide ion (Gupta & Sandalio, 2012).

[25] Transcription is the process where genes (DNA) are copied into transcripts (mRNA), which are then subsequently read (translated) by ribosomes into polypeptides (protein).

copper caused decreases in hemocyanin[26] and chitinase[27], interrupting molting and reproduction (Hook et al., 2014).

Jorge et al. (2013) noted that copper toxicity in organisms can be due to a range of dysfunctions, including ROS, disturbance of ion regulation, disturbance of acid-base balance, and impairment to ammonia excretion. In the case of the freshwater mussel, *Lampsilis siliquoidea*, mortality due to copper occurred when researchers exposed them to 2 µg Cu/L water for 28 days, a concentration below US EPA water quality standards (Jorge et al., 2013).[28] Based on various biomarkers, Jorge et al. speculated that copper toxicity in this case was not directly due to ROS. Instead, they concluded that copper was directly inhibiting cellular sodium-potassium ion pumps (Jorge et al., 2013). Besides being responsible for osmoregulation (ion balance), these pumps are also responsible for ATP production (Lodish & Darnell, 1995).

Copper ion toxicity in fish

Copper disrupts the function of fish gills (Eisler, 1998). Once in the gill, copper binds to cellular components and can:

- block functional groups of biologically important molecules
- displace other metal ions that are critical to proper function
- change the shape of biologically important molecules, disrupting their function

Together, these actions inhibit the transport of ions across membranes, and impair osmoregulation[29]. This can lead to a reduction in oxygen transport through the animal, lysis (breakdown) of cell membranes, and necrosis (cell death due to injury) of tissue in organs like the liver (Eisler, 1998).

Copper ion toxicity in mammals and birds

In mammals, excess copper absorbed in gastrointestinal cells is bound by special detoxifying proteins, and eventually removed when intestinal cells slough away (Eisler, 1998). Some copper is absorbed through the gastrointestinal tract, but this is regulated according to copper levels in the body. However, copper that enters the body through epithelial cells (e.g., the respiratory system, skin) can then move to the blood stream. This is the most likely pathway for toxic concentrations of copper in mammals. Copper at toxic levels can create ROS, interfere with enzyme functioning, and cause damage to membranes—effects similar to those seen in other organisms (Eisler, 1998).

Copper toxicity in wild birds and mammals occurs rarely because they are relatively resistant to copper at levels that are typically encountered (Kalisińska, 2019). Reports of toxicity exist for swans, geese, and red foxes in highly polluted areas. At high levels of exposure (such as food with more than 500 ppm Cu), copper can cause the breakdown (lysis) of red blood cells, inhibit enzymes, inhibit oxygen consumption, and cause mitochondrial swelling. Additionally, high levels can have a mutagenic effect on mammalian cells (Kalisińska, 2019).

Copper toxicity in amphibians and reptiles

The mode of action for copper toxicity in amphibians and reptiles is assumed to be similar to that in fish, where copper impairs gas exchange and osmoregulation at the gills (Flynn et al., 2015). Elevated copper levels in amphibians can cause mortality, deformities, decreased body size, delays in metamorphosis, and alterations of behavior. Delays in metamorphosis can be especially damaging to amphibian populations because they often live in ephemeral bodies of water. If water dries up before the animals undergo metamorphosis, an entire generation may die (Flynn et al., 2015).

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[26] Hemocyanins are proteins analogous to hemoglobin, which carries oxygen in invertebrates.

[27] Chitinase is the enzyme responsible for degrading the cytoskeleton of insects, prior to molting (Kumar & Gong, 2018).

[28] USEPA BLM-derived water quality criteria of 2.18 µg Cu/L or the USEPA hardness-derived water quality criteria of 12.16 µg Cu/L (Jorge et al., 2013).

[29] Osmoregulation relates to the control of solutes (substances dissolved in a liquid) on one side of a membrane, in order to maintain a desired fluid pressure. This takes advantage of osmosis, where water moves across a membrane according to a concentration gradient.

Amphibians are generally more affected by chronic copper exposure than short-term acute exposure, but vary widely in response (Azizishirazi et al., 2021). Acute toxicity ranges from 17–2996 µg/L, depending on amphibian species. Chronic toxicity occurs between 0.38–500 µg/L, again depending on species. For acute toxicity, late embryonic and early larval periods are the most sensitive. Data on the sensitivity of different life stages to chronic copper exposure is minimal, but indicate that again, embryonic and larval stages were most susceptible. At sub-lethal concentrations, amphibians attempt to avoid areas with copper contamination. They also can have reduced responses to predators (even at low environmentally relevant concentrations of around 5 µg/L), making them more likely to be successfully attacked (Azizishirazi et al., 2021).

Copper can also damage animal reproductive systems. Experiments with Chinese soft-shelled turtles indicate that copper negatively affects the reproductive systems of male turtles at sub-lethal doses (Yang et al., 2022). Turtles injected with 5 mg/kg bodyweight copper sulfate showed a nearly 60% reduction in sperm viability, and at 50 mg/kg bodyweight, showed over 80% reduction in viability (18.2%) after 72 hours compared with controls. Reproductive tissues (epididymis) exhibited damage, and the researchers concluded this was due to a loss in membrane integrity due to the effects of copper. The researchers also observed a large increase in the production of ROS with copper treatments, leading to irreversible loss of sperm motility (Yang et al., 2022).

Copper ion toxicity in microorganisms

In microorganisms, copper toxicity is due to many of the same mechanisms described for other organisms, such as (Trevors & Cotter, 1990; Avery et al., 1996):

- creation of ROS
- disruption of proteins (including enzymes)
- disruption of plasma membranes
- inhibition of respiration (O_2 consumption)
- displacement of essential ions

Avery et al. (1996) found that the composition of the membrane was associated with copper sensitivity. Yeast (*Saccharomyces cerevisiae,* a fungus) typically have membranes composed of saturated and monounsaturated fatty acids.[30] However, when forced to take up polyunsaturated fatty acids into their membrane, yeast cells became more susceptible to copper toxicity. The results of this study indicated that ROS (created by copper) target polyunsaturated fatty acids. Additionally, some degree of microbial susceptibility may intrinsically relate to membrane composition (Avery et al., 1996).

In a review of heavy metal toxicity literature, Bååth (1989) determined the following order for the toxicity of metals on microorganisms (from most to least): cadmium>copper>zinc>lead. Researchers have found nitrogen fixation to be very sensitive to copper contamination, even at low levels (~15 ppm EDTA extractable Cu).[31] Microorganisms can also have lower activity in copper contaminated soils, producing lower quantities of enzymes (Bååth, 1989).

<u>Evaluation Question #6:</u> Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

Copper and copper products cause many forms of contamination and environmental damage during manufacturing, waste disposal, and use.

Manufacture, mining:

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[30] Saturation refers to the degree to which fatty acids are hydrogenated or bonded to hydrogen. Fatty acids are chains of carbon atoms, with hydrogen atoms bonded to all other available sites. A fatty acid that is fully saturated, cannot be bonded to more hydrogen, and typically maintains a straight shape. A monounsaturated fatty acid is missing a single hydrogen atom and becomes kinked (or bent) in one spot. A polyunsaturated fatty acid is missing two or more hydrogens atoms and develops additional kinks.

[31] EDTA extractable Cu is used to assess copper bioavailability (Brewin et al., 2007)

Copper ore is mined using either underground, or open-cast processes (Lossin, 2001). Open copper mining pits are the largest ore mines on Earth, covering more than one square kilometer, and are hundreds of meters deep. Copper is also extracted through leaching, where sulfuric acid is used to dissolve low-grade copper ore in place, sometimes in conjunction with blasting to increase the available surface area (Lossin, 2001).

Heap leach mining involves making a pile of crushed ore, and then spraying a solution of sulfuric acid on top (Lossin, 2001). Of the leaching methods, heap leaching is the primary method (EPA, 2015). For every ton of copper, 1.7 tons of sulfuric acid are needed (Lossin, 2001). Heap leaching presents significant risks of environmental contamination through the leakage of toxic acids and metals (Petersen, 2016). Though heaps are contained by liners, the risk of liner leaks is considerable (Petersen, 2016). Spent heaps are also sometimes removed from the lined system and added to waste piles despite not being completely exhausted, leading to slow leaching of the remaining metals by acid, and the release of toxic components into the environment (Petersen, 2016). Wind-blown acid from spraying heaps is also a drift concern (Petersen, 2016).

Other leaching processes use one or more of the following (Lossin, 2001):

- ammonia
- iron chloride
- sodium chloride (salt)
- sodium bromide
- calcium oxide
- cyanide (for gold recovery)
- heat-resistant bacteria.

Leach purification processes use (Lossin, 2001):

- kerosene
- petroleum-based extractants
- sulfuric acid (during electrowinning).

Once copper ore is obtained, additional chemicals may be employed to refine it. Xanthanates, alcohols, sulfuric acid, fatty acids, and sodium hydrogen sulfide, are used in various copper ore refining processes (Lossin, 2001).

Mining and processing copper ore exposes and concentrates naturally occurring radioactive materials (primarily uranium and radium), referred to as “Technologically Enhanced Naturally Occurring Radioactive Materials,” or TENORM (EPA, 2015).

Accidental or intentional releases of toxic chemicals such as sulfuric acid or manufacturing wastes are not uncommon during mining. The following is a small selection of contamination events, related to copper mining:

- 1989, Panguna mine, Bougainville Island, Papua New Guinea (Doherty, 2021). One billion metric tonnes of mine waste was dumped into the Kawerong-Jaba river delta. Local citizens led a revolt against the mine due to the catastrophic environmental contamination and inequitable division of profits. After a decade of war between citizens and the government effort to restart the mine, 20,000 people were killed (Doherty, 2021).
- 2009, CopperCo Lady Annie mine, Queensland, Australia (Latimer, 2012). An uncontrolled release of contaminated water extended 52 kilometers into stormwater ponds and creeks, killing crabs and fish (Latimer, 2012).
- July 3, 2010: Zijinshan Copper Mine, Fujian, China (Reuters, 2010). Over 9,100 cubic meters (9 million liters) of copper mine wastewater was released into a river, killing 1.9 million kg of fish. Forty percent of soils in the region are contaminated by heavy metals, partly caused by 3,000 mines in the area (Reuters, 2010).
- August 7, 2014: Buenavista copper mine, Cananea, Mexico (BBC News, 2014). Forty thousand cubic meters (thirty-seven million liters) of copper and sulfuric acid was released into Bacanuchi river

(BBC News, 2014). The spill caused health, economic, and environmental problems for local communities, including suspected metals contamination in children (lead, aluminum) and cancer (Blust, 2019a). The same mining company (Grupo México) has been involved in numerous toxic accidents, including spilling 3,000 liters of sulfuric acid into the Sea of Cortez (Blust, 2019b).

- Various dates, including as recent as 2019: United States operating copper mines (Bonnie Gestring, 2019). Using government and industry documents, Earthworks (a nonprofit organization) found that 14 out of 15 open-pit copper mines in the U.S. "failed to capture and control wastewater, resulting in significant water quality impacts" (Bonnie Gestring, 2019). Their report includes a table of mine failures, due to a variety of causes (there was no one single issue).
- 2016: Berkeley Pit, Butte, Montana: 3,000 snow geese died after landing on water in the open pit copper mine, contaminated with acid and heavy metals (Gammons & Icopini, 2020). In the past, the pit has had a pH as low as 2.7, with thousands of mg/L of total dissolved metals (Gammons & Icopini, 2020). The Berkeley Pit is part of the largest EPA Superfund site in the United States (EPA, 2016b; Gammons & Icopini, 2020). Water in the pit is contaminated with a wide array of metals and other chemicals such as pentachlorophenol (EPA, 2022b; Gammons & Icopini, 2020).[32]

Manufacture, smelting of ore to matte and slag:
Many copper roasting, smelting, and refining processes use fossil fuels such as natural gas, coal, or coke as a fuel and/or as a reducing agent (Lossin, 2001). Copper production currently accounts for 0.3% of total greenhouse gas emissions (Watari et al., 2022). However, due to increasing demand, copper production could account for approximately 2.7% of total greenhouse gas emissions by 2050 (Watari et al., 2022).

Copper ores can contain high concentrations of arsenic and antimony (Lossin, 2001). When these ores are processed, a material called speiss is formed, which contains these elements (Lossin, 2001). Arsenic inhibits enzyme functions, and can poison skin, the kidneys, and the nervous system (Sherameti & Varma, 2010). Antimony can cause issues related to breathing, blood pressure, cardiac function, ulcers, loss of reproductive capability, and cancer (Sundar & Chakravarty, 2010).

Flue dust from copper smelting is toxic (Lossin, 2001). Flue gases and dust from copper furnaces contain (Lossin, 2001):

- sulfur dioxide
- sulfates of copper, lead, and zinc
- volatile compounds of arsenic, antimony, bismuth, and selenium

Manufacture, conversion of matte to metal:
During the conversion process, some equipment can allow sulfur dioxide (SO_2) gases to escape (Lossin, 2001). In some cases, secondary hooding systems are used to collect fugitive gases, which are then converted into gypsum (Lossin, 2001).

Manufacture, recycling of scrap:
Depending on the purity, scrap may need to undergo a smelting process, where coke or natural gas are used (Lossin, 2001). Copper recovery from circuit boards leads to the production of carbon dioxide (CO_2), as the organic polymers in the board material are incinerated (Lossin, 2001).

Manufacture, refining:
Copper refining involves the use of natural gas, sulfuric acid, ammonia, carbon monoxide, propane, diatomic hydrogen and nitrogen gas (Lossin, 2001).

Manufacturing, overall:
For every kilogram of copper, 0.8 to 1.6 kg of sulfur are produced, typically in the form of sulfur dioxide gas (Lossin, 2001). The sulfur dioxide produced from copper manufacturing contributes to acid rain (Naeem et al., 2020).

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[32] Pentachlorophenol is an industrial wood preservative, used to treat poles, posts, and bridge timbers at the Montana Pole and Treatment Plant, another nearby Superfund site (EPA, 2022a).

Large amounts of water are needed for cooling furnaces and other high-temperature handling equipment. Water used in hydrologic processes such as leaching must be treated with lime (calcium oxide) in order to neutralize and precipitate sulfur and heavy metal ions (Lossin, 2001). Between 2004–2008, the average annual water usage for all copper mines in Arizona was 55,659 acre-feet, or roughly 69 billion liters (Singh, 2010). For comparison, this amount of water is roughly 4.5% of that withdrawn for domestic use in Arizona in the year 2000 (Konieczki & Heilman, 2004). The average water usage per pound of copper in Arizona varied between 5.4-54.6 gallons (45–456 liters of water per kilogram of copper) (Singh, 2010). More recently, it was estimated that the proposed Resolution copper mine near Superior, Arizona, will use 500,000 acre-feet per year,[33] or 18% of Arizona's supply from the Colorado River (Abbott, 2021).

According to Richardson (2000), reclamation and recycling of copper wastes is both environmentally and economically important. This practice has reduced the amount of copper released *directly* into waterways.[34] Copper is reclaimed from the electronics, electroplating, and copper refining industries, and subsequently converted into materials such as agricultural-grade copper(II) sulfate. A variety of chemicals are used to isolate and convert these wastes, including neutralizing agents, sodium hydrogen sulfite, sodium hydroxide, formaldehyde, or sodium dithionite (Richardson, 2000).

Manufacturing, Copper Compounds

All of the following materials in Table 11 can be produced through different processes and, therefore, may use a variety of ingredients and processing aids. Many of these substances could be toxic if released or mishandled.

Table 11: Copper compounds and the substances they are manufactured from. Information taken from Richardson, 2000.

Copper substance	Manufactured using:
copper(II) sulfate	copper metal, alkaline ammoniacal copper(II) solutions, sulfuric acid, calcium oxide, calcium stearate, etching wastes, and copper sludge (specifically used for agricultural purposes)
copper(II) sulfate basic	copper(II) sulfate, sodium carbonate, copper(I) oxide, sulfuric acid, ammonia, ammonium sulfate, trisodium phosphate, sodium hydroxide, and calcium oxide
copper(II) hydroxide	copper(II) ammine complex, sodium hydroxide, basic copper(II) carbonate, copper(II) phosphate, copper(II) oxychloride, anionic surfactants, magnesium sulfate, and trisodium phosphate
copper(II) oxide	copper metal, copper(II) nitrate trihydrate, basic copper(II) carbonate, copper(II) carbonate, ammonia ,ammonium carbonate, salts (strontium, barium, calcium), copper(II) amine sulfate, and recycled copper(II) amine chloride
copper(I) oxide	copper ore or metal, sulfur dioxide, sodium sulfite, sodium hydroxide, isophthalic acid, pine oil, glues, gelatin, casein, and dextrin
copper(II) oxychloride	is produced using copper(i) chloride, sodium chloride, and sodium hydroxide
copper(II) carbonate hydroxide	copper(II) sulfate, sodium carbonate
copper(II) ammonia complex	can use copper(II) salts, ammonia, ammonium sulfate, and sodium hydroxide
copper salts of fatty and rosin acids	kerosene, copper(II) hydroxide, basic copper(II) carbonate, copper(II) sulfate, and sodium salts of fatty acids

Agricultural use

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[33] This is about equal to the water needs of one million people, for one year (Abbott, 2021).

[34] Recycled copper is used to create agricultural-grade copper sulfate, and therefore these products are released into the environment (including waterways), just at a later time.

Copper concentrations in soil vary due to geologic and soil-forming processes (Panagos et al., 2018). However, the influence of humans on copper concentrations in soil has now become stronger than these natural factors. Excessive use of copper fungicides, bactericides, and other pesticides has contributed to copper accumulation in agricultural soils, especially vineyards (Adrees et al., 2015). According to Alloway (2013), average application rates vary between 2-4 kg Cu/hectare/year. However, in specialty systems such as viticulture, copper can be applied at rates approaching 80 kg Cu/hectare/year (Lamichhane et al., 2018). Citrus and avocado orchards also use high rates of copper, sometimes receiving 15 applications per year (Lamichhane et al., 2018). We consider this number of applications noteworthy, because in the EPA's 2009 *Reregistration Eligibility Decision for Coppers*, the EPA assumed a total of four copper applications per year (EPA, 2009).

Vineyards, olive groves, and fruit orchards in Europe have a higher average concentration of copper than surrounding areas (Panagos et al., 2018). Fifteen percent of European vineyards have soil copper concentrations greater than 100 mg/kg soil (Yu et al., 2022). In Australia, vineyards also show elevated levels of soil copper as compared with nearby areas, though not as extreme as in Europe (Wightwick et al., 2008). In Brazil, Hummes et al. (2019) found that in 100-year old vineyards, bioavailable soil copper reached 1100 mg/kg soil. This led to 12,300 ppm Cu dry weight in grapevine roots, and 6,800 ppm Cu dry weight in grape leaves. Juice made from these grapes contained 0.08 mg Cu/L, 908% above the limits set by Brazilian and international norms (Hummes et al., 2019).

During the early 1900s, copper fertilizers (at a rate of 34 kg Cu/hectare/year) and fungicides (10kg/hectare/year) were applied to citrus groves in Florida (Eisler, 1998). Surface soils in these areas now contain 540 kg Cu/hectare (Eisler, 1998). More recent studies have found other agricultural locations in Florida with concentrations as high as 1200 ppm Cu (Hoang et al., 2008). See Table 12 (below) for more examples of elevated levels of copper in agricultural soils.

A study by Melendez et al. (2020) of ten organic and five conventional vegetable farms in New Jersey found that copper fungicide use had increased the level of copper in soils. The average background level of copper in New Jersey rural areas was 4.8 ppm. On the studied farms, areas where copper was not historically applied had an average soil copper level of 10.2 ppm, whereas the average in areas where copper was applied was 32.1 ppm. One farm had a soil copper level of 153 ppm and another over 48 ppm (Melendez et al., 2020).

Table 12: A selection of reports of soil copper concentration. Uncontaminated soils generally have <20 mg Cu/kg (ppm) soil, but levels can be as high as 100 mg/kg in some instances. Adapted from Lamichhane et al., 2018.

Continent	Country	Type of crop	Total copper, ppm
Asia-Pacific	Australia	Vineyard	63–340
	New Zealand	Vineyard	1–259
	New Zealand	Stone fruit orchard	21–490
	Taiwan	Vineyard	9.1–100
Africa	South Africa	Vineyard	10–20
	Tanzania	Coffee orchard	24–366
Europe	France	Vineyard	57–1500
	Italy	Vineyard	93–478
	Portugal	Vineyard	8–574
	Serbia	Vineyard	24–432
	Slovenia	Vineyard	65–120
	Spain	Vineyard	41.5–583.1
North America	USA	Vineyard	87–142
	USA	Citrus orchard	Up to 250
South America	Brazil	Vineyard	36–3215+

In China, increases in soil copper due to agricultural use have also been documented (Niu et al., 2013). Pig and poultry manure containing excess copper are made into slurries and used as fertilizers, which then contaminate livestock grazing land (Adrees et al., 2015). Livestock manure may account for 69% of the soil copper input in China (Niu et al., 2013).

The CDC notes that copper can enter the environment from mining, farming, manufacturing, and wastewater releases (CDC, 2018). Copper can enter drinking water directly through contaminated wells, or through corrosion of copper pipes with acidic water sources. They also note that corrosion of pipes is "by far the greatest cause for concern."

For more information on manure as a source for copper, see *Focus Question #2*.

Evaluation Question #7: Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

The following reactions are noted in literature:

- Copper sulfate (and presumably other sources of copper(II)) was recently shown to oxidize organic matter in soil to produce volatile halogenated hydrocarbons such as methyl bromide and methyl chloride (Jiao et al., 2022). Soils can act as either sinks or sources of these ozone-depleting substances (Jiao et al., 2022). Adding hydrogen peroxide amplifies this effect, between 7 and 93-fold, depending on the halogenated hydrocarbon of interest. In nature, H_2O_2 is produced by exposing humic substances to sunlight. Experiments in soil containing humic substances showed that sunlight increased the effect, as was predicted (Jiao et al., 2022).
- Copper^{+1} (cuprous) ions can react with iodine (forming CuI), sulfur (forming CuSCN), and certain nitrogen-based complexing agents (Lossin, 2001).
- Copper^{+2} (cupric) ions can react with oxygen (forming molecules like $[Cu(H_2O)_6]^{2+}$) or nitrogen-based complexing agents, such as ammonia (forming copper ammonia complexes). In the presence of oxygen, copper dissolves in ammonia and acetic acid and forms verdigris, a green pigment (Lossin, 2001). We do not expect that the formation of these substances during organic production would be significant.
- Copper ions can also inhibit the uptake of zinc by plants (Gowariker et al., 2008).
- Copper sulfate is a strong oxidizing agent (Beer et al., 1998). It can corrode iron and galvanized iron (Beer et al., 1998).
- Copper salts should be stored away from acetylene gas (which may be present in welding equipment), because it reacts to form the substances CuC_2 and Cu_2C_2, which are explosive (Lossin, 2001).
- Copper(I) oxide and copper(II) chloride act as a catalyst for numerous chemistry reactions (Richardson, 2000).
- Water-soluble chelating agents such as fulvic acids can reduce the acute toxicity of copper ions to some animals, such as marine copepods, through complexation, leading to a reduction in bioavailability (Eisler, 1998).

Other ingredients used in copper products can change their toxicity (Closson & Paul, 2014). Most studies on copper products focus on active ingredients. Closson & Paul (2014) tested formulated products, and found that:

- Surfactants added to copper carbonate increased copper toxicity to fish, compared with copper sulfate.
- Chelating agents altered toxicity in different ways, depending on the fish species.
    - In fathead minnows, the formulation with a chelating agent was less toxic than copper sulfate.
    - In young brook trout, the same product was equally toxic to copper sulfate.
    - In larger brook trout, it was initially less toxic than copper sulfate, but over time became more toxic.

Bordeaux mixture will be discussed separately in *Evaluation Question #10* (below).

<u>Evaluation Question #8:</u> Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

While an attempt was made to separate responses in *Evaluation Question #5* and *#8*, these questions are interrelated. Copper products negatively affect the survival and behavior of many soil and aquatic organisms. Descriptions of this, including the mode of action, are included under *Evaluation Question #5*. Here, we will focus on the result of the effects of copper on organisms, and the known incidents or potential for incidents to occur within the agro-ecosystem.

Ecosystems

In a study in Denmark, researchers selected an area with a copper gradient from 22 ppm Cu, to 3800 ppm (Naveed et al., 2014). Copper sulfate had been applied in the area between 1911 and 1924 as a wood preservative. Since 1993, the field had been fallow, and copper was the only contaminant found in the soil. Researchers measured earthworm density, plant species richness, DNA (for microorganism identification), and soil characteristics. As copper concentration increased (Naveed et al., 2014):

- density and biodiversity of plants decreased, becoming observable at ≥ 200 ppm Cu
- density and number of species of earthworms decreased, along with changes to the relative abundance
- decrease in the richness and diversity of other organisms, including nematodes (>175 ppm Cu) and bacteria (>560 ppm Cu)
- changes in the community composition of fungi
- soil became more compacted due to a loss of bioturbation (mixing/aeration) by earthworms and plants
- soil pore size decreased
- gas transport decreased due to decreased soil aeration
- organic matter increased, due to a loss of decomposers
- soil water content increased

At sampling sites with 3837 ppm Cu, the researchers found the soil to be functionally "dead" (Naveed et al., 2014). The critical concentration corresponding to a 10% loss of soil biological, physical, and chemical indicators was:

- 110 ppm Cu soil for earthworms
- 170 ppm Cu soil for bacteria
- 800 ppm Cu soil for fungi, which were the most resistant organisms in the study.

This study is significant, because it shows negative effects of copper, applied over one century ago, at concentrations comparable to those found in a variety of agricultural soils.

Soil microorganisms

Microorganisms can develop a tolerance to copper over time, but often this comes with a change in the structure of microbial communities, including lower genetic diversity, changes to species composition, and lower overall fitness (Klimek & Niklińska, 2007; Bååth, 1989). Heavy metal contamination, including copper, tends to shift bacteria towards more gram-negative species (Bååth, 1989). Studies conflict as to whether fungi or bacteria are more sensitive to copper (Klimek & Niklińska, 2007).

There are limits to microbial tolerance. Copper contamination near smelters can be so significant that deep layers of organic matter accumulate due to the lack of microorganism decomposers (Giller et al., 1998). Forests are often more sensitive to copper contamination than agricultural soils, showing larger changes in organic matter cycling when contaminated (Giller et al., 1998).

Researchers in Australia used a field site with a known history to look at long term effects of copper exposure (Shaw et al., 2020). The plots were dosed with different concentrations of copper in 2004. Between 2004 and 2016, the total copper concentrations at individual field plots did not change. In 2016, the researchers measured various soil parameters and found that copper created a selection pressure for microbial species, resulting in changes to microbial communities.

- Based on the respiration rate of the soil, the EC50 for soil microorganisms was 483 ppm Cu.[35] In this soil, 385.4 ppm Cuwas considered bioavailable.
- Based on an analysis of the production of amino acids from the breakdown of proteins, the EC50 for nitrogen cycling was 663.4 ppm Cu. Above 1000 ppm Cu, nitrification processes ceased.
- Microbial communities in soil plots with <100 ppm Cu had highly variable community structures. In other words, separate field plots at and below this copper concentration were composed of communities with unique assemblies of species (high beta diversity).[36]
- Microbial communities in soil plots between 200 and 700 ppm Cu had microbial communities that were more similar from plot-to-plot (lower beta diversity). These plots were dominated by *Candidatus koribacter*, common in other areas contaminated with iron and uranium. *Burkholderia* spp. were also common.
- Microbial communities in soil plots with >800 ppm Cu had even more uniform communities (low beta diversity) compared with plots with lower copper concentration. These plots were dominated by resting cyst and biofilm forming bacteria, such as those in the Chitinophagaceae family. *Myxobacteria* spp. were also common.[37]

Copper has been found to decrease mycorrhizal associations with plants. In one study, copper decreased the colonization of citrus seedlings by mycorrhizae, with effects beginning at 19–34 mg/kg soil (Bååth, 1989). Mycorrhizae, though, may also provide plants with a degree of protection from heavy metals, including copper (Bååth, 1989).

Researchers have demonstrated that exposing soil microorganisms to copper causes them to develop resistance not only to the metal, but also to antibiotics such as chloramphenicol, tetracycline, and ampicillin (Yu et al., 2022). Additionally, long-term copper contamination in soils is associated with an increase in the occurrence of genes responsible for antibiotic resistance. Researchers working with several human-pathogenic strains of *Escherichia coli* K12 found that copper hydroxide applied at 100 and 200 mg/L caused:

- most strains to develop resistance to chloramphenicol, and tetracycline
- some strains to develop statistically significant resistance to erythromycin, ciprofloxacin, and azithromycin
- some strains to develop a higher average resistance to amoxicillin, but not a statistically significant difference
- no strains to develop resistance to kanamycin or vancomycin.

More concentrated applications of copper hydroxide (400 mg/L) impeded bacterial growth rates enough that antibiotic resistance was not able to develop, and lower concentrations (10 mg/L) were insufficient to cause sufficient selection pressure. After 150 generations, many of the bacteria retained this resistance (Yu et al., 2022).

Soil and other macroorganisms

Scientific literature describing the effect of copper on non-target soil macroorganisms in agricultural or other field settings is limited. Literature describing toxicity in lab experiments is discussed in *Evaluation Question #5*. Copper is toxic to a range of animals at levels relevant to agricultural applications, including nematodes, segmented worms, and arthropods (Alloway, 2013).

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[35] The EC50 is the concentration of a substance that produces a 50% response. In this case, a 50% reduction in activity.

[36] There are different types of biodiversity. The number of species (species richness) at an individual location (or habitat) is a measure of alpha "diversity" (Morin, 1999). Measuring how many species are different between a variety of similar locations in a region is called "beta" diversity (Morin, 1999). Beta diversity is somewhat analogous to variance.

[37] *Myxobacteria* spp. are considered "micropredators," capable of killing prey by secreting substances that kill and digest a variety of prey, including other bacteria, yeast, fungi, protozoans, and nematodes (Dai et al., 2021)

Restoration of wetland ecosystems in the Florida Everglades has been affected by historic applications of copper to citrus orchards (Hoang et al., 2008). The Florida apple snail (*Pomacea paludosa*) is a key species, which other birds, fish, reptiles, and mammals rely on as a food source. Restoration of wetland ecosystems in Florida involves flooding agricultural soils. Some of these soils are contaminated with high levels of copper. These soils can release copper into water at levels significantly higher than limits set by EPA[38], capable of damaging aquatic organisms. In one study, researchers measured the desorption of copper from soils, as well as the effect of this on the Florida apple snail. A selection of sites with a range of copper concentration up to 204 ppm Cu (dry weight) was used. Two sites, originally with soil copper concentrations of 174 and 204 ppm Cu, released enough copper into water when flooded that they would theoretically produce adverse effects on freshwater algae, rotifers, mollusks, crustaceans and fish. Florida apple snails grown on flooded soil from these same two sites showed a drop in survival of 47% and 60%. Copper was believed to accumulate in the snails through exposure to soil, water, and through diet. This result has implications for species dependent on the snail for survival, including endangered species (Hoang et al., 2008)

Copper can also affect the behavior of invertebrates in ecologically significant ways (Hunting et al., 2013). Soil and aquatic invertebrates contribute to decomposition, vital to ecosystems (including organic crop land). Their movement, feeding, and defecation alters sediment texture, disturbs solid particles, and oxygenates soil. This activity benefits aerobic microorganisms that contribute to decomposition as well. In laboratory experiments, copper was shown to have a strong effect on the behavior of two invertebrate species (*Tubifex* worms and *Asellus aquaticus,* a.k.a. water louse). Increasing concentrations of copper (range of 5.6–487.6 ppm Cu dry weight sediment) reduced the mixing/disturbance of the sediment due to invertebrate movement, limiting their activity to just the upper surface (Hunting et al., 2013).

Similar studies have indicated that earthworms also exhibit avoidance behavior to copper-contaminated soil (La Torre et al., 2018).

Plants

The negative effects of excess copper on plants are well studied. At higher concentrations, copper is highly toxic to plants, more so than manganese, zinc, and cadmium (Adrees et al., 2015). In a review of the effects of copper on plants, Adrees et al. (2015) summarized studies showing that the effects of excess copper decreased germination and biomass in a wide variety of crops, and negatively affected various aspects of plant morphology. They also noted that copper toxicity in plants commonly causes a decrease in the uptake of other mineral nutrients, including phosphorous, zinc, iron, and manganese (Adrees et al., 2015).

Excessive soil copper concentrations can have fertilizer management implications. For example, as little as 20 ppm Cu in sandy soil can cause potassium, magnesium, and calcium to decrease in cucumber leaf tissue (Adrees et al., 2015).

Prior to 2009, the EPA had been notified of the following incidents from the use of copper pesticides (EPA, 2009):

- One report of damage to tomatoes in Washington, when copper was used according to label directions.
- Six incidents of damage to corn and peanuts in Indiana, Minnesota, and Oklahoma. The incident reports did not include whether the pesticides were used properly.

Aquatic organisms

Prior to 2009, the EPA had been notified of 17 incidents where aquatic organisms were killed from the use of copper pesticides (EPA, 2009). The EPA noted that 10 of the incidents occurred even though the operator likely followed the directions on the registered product label. According to the EPA, use of copper

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[38] EPA water quality limit for freshwater organisms is 13 µg/L, while Hoang et al. (2008) estimated that these Florida soils could release enough copper to raise the concentration to 118–3015 µg/L.

pesticides in these cases generally killed 100–1,000 fish, with the exception of one incident in New York where over one million fish were killed according to the EPA report.[39]

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

The manufacturing, disposal of manufacturing wastes, use, and misuse of copper compounds are all harmful to the environment. Manufacturing copper results in long-lasting environmental degradation due in part to leaks and spills of toxic chemicals—especially sulfuric acid and other heavy metals. Copper manufacturing consumes large amounts of water, often in arid areas susceptible to drought. Copper manufacturing produces greenhouse gas emissions, which are likely to increase as the demand for copper increases as well.

Mining also disrupts the land itself. In some cases, copper mining activities are so destructive that areas require federal funding in order to be cleaned up, taking decades or longer. These areas are identified on the National Priorities List, also known as "Superfund" sites. According to a 2004 report from the U.S. Department of Health and Human Services, "copper has been identified in at least 906 of the 1,647 hazardous waste sites proposed for inclusion on the EPA National Priorities List" (Agency for Toxic Substances and Disease Registry (ATSDR), 2004).

The use of copper products in agriculture has also contributed to contamination of soil and water. This contamination occurs at environmentally significant levels, causing harm to a wide variety of organisms, including bacteria, fungi, invertebrates, fish, and plants. In some cases where copper fungicides have been used for prolonged periods of time, soil copper can reach levels similar to that found around copper smelters, on the order of thousands of ppm (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). Copper contamination can disrupt ecosystem community structure and the behavior of organisms, sometimes resulting in physical changes to soil structure and composition itself. Site-specific variables including the history of total copper accumulation (from all sources), soil characteristics, and the crops and other organisms present will all affect whether or not use of copper fungicides will exceed critical levels that cause harm. Because of this complexity, it is not possible to simply use label rates as a guide to evaluate copper fungicide environmental safety at every given location.

In addition to soil and water, application of copper(II) pesticides may increase ozone-depleting chemicals in the atmosphere. Copper(II)-mediated production of methyl bromide and methyl chloride in the soil is estimated to be responsible for 4.1 ± 1.9 million kg per year and 2.5 ± 0.7 million kg per year respectively (Jiao et al., 2022).

Information detailing the effects described above are discussed *Evaluation Questions #5-8*. Additional information is presented below regarding EPA and EFSA's evaluations of copper products for pesticidal use. Both of these agencies found that use of copper products pose inherent risks to the environment.

In 2005, the EPA's ecological risk assessment for the use of copper products indicated "significant risk exceedances for virtually all non-target organisms" (EPA, 2009). The EPA subsequently gathered use data

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[39] The authors of this report find this data point confusing. The only public records we found that relate to copper-caused fish deaths in New York State of this magnitude were non-pesticidal in nature (Associated Press, 2001; Nolan, 2008). Two incidents occurred at the Oak Mitsui (a circuit board facility) in Hoosick Falls, NY: one in 1983, and the other in 2001 (Nolan, 2008).

- The exact source and substance spilled in 1983 was difficult to identify, but was attributed to the circuit board facility, and the substance increased the copper concentration to 38.7 ppm copper, killing 100,000 white suckers, minnows, and trout (Barbanel, 1983).
- Approximately 2,000 gallons of sulfuric acid were spilled from the facility into the Hoosick river in 2001 (Associated Press, 2001). Hundreds of thousands of fish were reported killed.
- Additionally, in 2006, water near the same facility showed similar water quality changes, but no incident was reported (Nolan, 2008).

from growers and other community groups, which they used in their subsequent 2009 *Reregistration Eligibility Decision (RED) for Copper*. In their *Executive Summary*, the EPA noted that they had revised their risk assessment, and indicated that use of copper pesticides carries the following risk:

- potential risk to terrestrial organisms
- low risk to terrestrial plants
- low risk to honeybees
- risk to other insects are unknown
- <1% of freshwater fish at risk to acute exposure; <1–5.3% of freshwater fish at risk to chronic exposure, depending on application rates
- 3.2–25% of aquatic invertebrates at risk to acute exposure; 4.2-32% of aquatic invertebrates at risk to chronic exposure, depending on application rates
- acute and chronic risk to freshwater non-vascular plants at relatively low application rates (1.5 lbs Cy/acre, or 1.7 kg Cu/hectare)
- acute and chronic risk to estuarine and marine animals at 3 lbs Cu/acre (3.4 kg Cu/hectare).

The EPA's evaluation of ecotoxicity was based solely on pesticide applications, and did not take into consideration compounded risks due to other agricultural or anthropogenic copper inputs (EPA, 2009).

At the time of the 2009 report, 24 ecological incidents had been reported to the EPA related to copper pesticides (EPA, 2009). Seven incidents were related to crop plants, while 17 incidents were associated with killing aquatic organisms, mostly fish (EPA, 2009). We found these incidents noteworthy, because the organisms damaged or killed in these cases are specifically those EPA considered at lower risk (freshwater fish and terrestrial plants). Based on the EPA's assessment of toxicity risk, we conclude that it is likely that incidents reported to have killed fish are likely to have also caused other unreported ecological damage, particularly to aquatic invertebrates and non-vascular aquatic plants. It is therefore also likely that incidents causing damage only to invertebrates, which are more sensitive and less visible or obvious, go unnoticed or unreported.

Through a FOIA request, we were able to acquire additional EPA ecological incident data covering the period between 2009 and early 2022. We have summarized this data from the Ecological Incident Information System (EIIS) in Table 13, below. Some of the information appeared to be repeated in reports. We eliminated instances that appeared to be duplicates in the raw data from the summary table. Incident report detail was limited. We have used the same coding as the EPA reports.

Table 13: Individual EPA incident reports for copper pesticides categorized by active ingredient (2009-2022). Summarized from data acquired through a FOIA request.

EPA PC Code	Active ingredient	HB	HC	HD	PA	WA	WB	DA	DB	DC	DD	Other
024401 024402 024408	Copper(II) sulfate (pentahydrate/monohydrate/ anhydrous)	12	516	2	3	3	11	2	7	54	34	59
008101	Copper(II) sulfate basic	-	-	-	-	-	-	-	-	-	-	1
023401 072598	Copper(II) hydroxide	2	35	2	2	1	-	-	-	-	-	97
025601	Copper(I) oxide	-	1	-	-	-	-	-	-	-	-	73
023501 008001 008101	Copper(II) oxychloride	1	22	10	-	-	-	-	-	-	-	5
023503	Copper(II) oxychloride sulfate	-	10	1	-	-	-	-	-	-	-	-

EPA PC Code	Active ingredient	HB	HC	HD	PA	WA	WB	DA	DB	DC	DD	Other
022901 072598	Copper(II) carbonate hydroxide	-	18	-	-	-	-	-	-	-	-	-
024407 039105	Copper(II) ethylenediamine complex	-	4	3	-	-	1	-	-	-	-	-
023306	Copper(II) octanoate	27	570	-	3996	-	-	-	-	-	-	2185
024403	Copper(II) triethanolamine complex	2	251	-	-	2	-	-	-	-	-	63
024409 024410	Copper(II) ethanolamine complex	1	278	1	-	4	28	-	-	-	-	54

HB: Human - Major [e.g., suicide attempts, respiratory distress, seizure, tremors, decreased heart rate, mental confusion, miscarriage, enlarged spleen, stroke, hypertension, pneumonia, chest tightness, low hemoglobin, dizziness, hallucination, loss of consciousness, coma, cancer, blood clots, jaundice, liver or kidney dysfunction, or chronic vomiting]
HC: Human – Moderate [e.g., coughing, rash, itching, blistering, vomiting, shortness of breath, nausea, stomach cramping, eye and nose irritation, chest pains, blurred vision, wooziness, gagging, sore throat, lethargy, joint pain, diarrhea, headache, or chemical burns]
HD: Human – Minor [e.g., eye irritation, skin irritation, or rash]
PA: Plant Damage – Major [e.g., crop loss, leaf burn, or yellowing]
WA: Wildlife – Major [e.g., major fish kill events]
WB: Wildlife – Minor [e.g., minor fish kill events, or fish gasping for air]
DA: Domestic Animal – Fatality [e.g., koi fish kill, or non-specific veterinary reports]
DB: Domestic Animal – Major [e.g., undifferentiatied veterinary reports of tremor, lethargy, or ulcers]
DC: Domestic Animal – Moderate [e.g., undifferentiatied veterinary reports of tremor, lethargy, or ulcers]
DD: Domestic Animal – Minor [e.g., undifferentiatied veterinary reports of tremor, lethargy, or ulcers]
Other: A number of incident reports describe undefined incidents, listed as “aggregate summaries,” non-specific human, plant, or animal cases, uncategorized new reports, “individual incident reports,” “other,” or “other Nontarget.” Bee kills are classified as “Other Nontarget” instead of wildlife-related incidents. Bee kill events are described for:

- copper(II) sulfate: 2
- copper(II) hydroxide: 4
- copper(I) oxide: 1

The European Food Safety Authority (EFSA) reviewed copper products in 2017 as a fungicide on grapes, tomatoes and cucurbits (EFSA, 2018). They concluded that using copper fungicides at approved levels posed a high risk for:

- birds
- mammals
- algae and aquatic organisms, when used on grapes
- soil macroorganisms such as earthworms
- bees (oral and contact) (some formulations of copper products under worst-case scenarios)
- non-target arthropods, except for the use of Bordeaux mixture and copper(I) oxide

However, EFSA (2018) noted that numerous data gaps existed for their review, including:

- peer-reviewed scientific literature from within 10 years of the report
- certain types of data on individual copper products
- data on copper residues on certain crops

- toxicology data on stabilizers used in copper products
- toxicity data to refine risk assessments for different types of organisms

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

According to the EPA (2009):

- "copper dietary exposures do not pose any risks of concern."
- "based on available literature and studies, there is no indication of systemic effects resulting from copper exposures."
- "copper is ubiquitous and naturally occurs in many food sources… Human have the capability to metabolize and regulate copper levels in the body."
- "since no systemic toxicological endpoints of concern were identified for dermal exposures to coppers, no dermal, oral or inhalation endpoints of toxicological concern were established"
- "Occupational and residential exposures to copper pesticides are not of concern to the Agency."

The EPA's EIIS database includes 45 major, 1,705 moderate, and 19 minor reported human health incidents related to exposure to copper pesticides occurring between 2009-2022 (Table 13, above). Empirical evidence therefore indicates that copper products can be hazardous to human health. Furthermore, the EPA does state that copper products do exhibit acute toxicity (EPA, 2009).

Between 1993–2003, 82 incidents were reported to the Poison Control Center due to copper products, mainly copper sulfate and copper hydroxide (EPA, 2009). Symptoms included eye irritation, nausea, vomiting, and skin irritation.

- Between 1982–2003, 156 out of 494 incidents reported to the California Department of Pesticide Regulation were due to copper compounds (EPA, 2009). "Symptoms included eye effects, skin effects, or other acute effects (i.e. respiratory effects)."
- Between 1984–1991, the National Pesticide Information Center (NPIC) received 15 reports of illness in humans due to copper hydroxide, and 13 due to copper sulfate (EPA, 2009).
- Between 1998–2003, 34 out of 5899 incidents reported to the National Institute for Occupational Safety and Health were due to copper products (copper sulfate pentahydrate, copper hydroxide, and copper ammonia complex) (EPA, 2009).

Regulation of copper levels in humans

Before discussing the specific health consequences of copper exposure, we present information below on how copper behaves in the body. Some of the following details will also be relevant for *Focus Question #1*.

As an essential element, small amounts of copper are required for cellular respiration, free radical defense, neurotransmitter function, connective tissue biosynthesis, and iron metabolism in humans (Harris & Gitlin, 1996). The recommended daily intake of copper is 1–2 mg/day, which is easy to attain in most diets (Alloway, 2013). The recommended dietary allowance established by the National Academy of Sciences ranges from 0.34 mg/day for children, to 1.3 mg/day for pregnant women (EPA, 2009). The European acceptable daily intake (ADI) for adults is 0.15 mg Cu/kg of bodyweight per day (EFSA, 2018). Meanwhile, Taylor et al. (2020) found that 0.04 mg/kg/day was sufficient for adults and children to prevent copper deficiency. For a 70 kg person, EFSA's recommendation is 10.5 mg/day, while Taylor et al. recommends only 2.8 mg/day.

In a typical diet, humans absorb on average about 36 % of the copper that they consume in food (EFSA, 2018).[40] EFSA estimated that through food, consumers were exposed to 72.3% of their ADI, while on average they received 15.1% of the ADI from drinking water. These exposures add up to 87.4% of the ADI recommended by EFSA, and exceed the quantity that was recommended by Taylor et al. (2020). According to the EPA, dietary copper does not pose a health risk. This includes both copper-rich foods such as meat,

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[40] This value depends on the copper status within the body. Humans vary in how much copper they absorb from diet, but it is typically between 20-60% (EPA, 2009).

nuts, and bread, and copper residues left from copper pesticides in crops (EPA 2009). However, research and case studies indicate that there are some situations in which this may not always be true (see *Health effects from exposure to elevated levels of copper*, below).

The same characteristics of copper that make it essential also make it potentially toxic—for example, its ability to easily facilitate the transfer of electrons (Harris & Gitlin, 1996). Copper binds to the amino acids histidine, cysteine, and methionine, disrupting their normal function (Prasad, 2008). Amino acids are the building blocks of proteins, and thus copper can disrupt proteins containing these amino acids. In order to combat potentially toxic quantities, the body regulates ingested copper levels as follows (Harris & Gitlin, 1996):

1. Copper is absorbed in the stomach and initial part of the small intestine (duodenum).
2. It is transported to the liver by albumin (a protein).
3. Once in the liver, copper is distributed evenly throughout the organ.
4. Copper is then exported to the bloodstream, where it is distributed to various organs to be incorporated into new proteins.
5. Homeostasis (balance) is maintained through the excretion of copper via the biliary system (bile ducts and organs such as the gallbladder) back into the gastrointestinal tract, where it is subsequently eliminated.

In a study using radioactive isotopes of copper, 10% was excreted in 72 hours, via urine and feces (Eisler, 1998). In four weeks, 50% was excreted. This study showed that copper retention time in humans is relatively high. Studies in rats show that biliary excretion of copper is associated with an increase in bile flow, increased body temperature, and production of adrenal steroids (Eisler, 1998). The body can also limit gastrointestinal absorption when exposed to doses above what is needed (Taylor et al., 2020). However, based on the existence of acute copper poisoning, there are limits on the mechanism by which the body prevents absorption. In addition, gastrointestinal absorption is not the only route of exposure for copper. Exposure through inhalation has been associated with various pulmonary issues, described later in this section.

In all vertebrates, 95% of copper in plasma is bound to the protein ceruloplasmin (Harris & Gitlin, 1996; Patel & Aschner, 2021). This protein is synthesized in the liver, and is bound to copper before it is secreted into the bloodstream. The purpose of ceruloplasmin is not for copper transport as has been suggested in the past, but instead it uses copper as a cofactor to assist with iron metabolism (Harris & Gitlin, 1996). Ceruloplasmin binds copper very strongly, and does not typically exchange it with other proteins or other molecules (Sternlieb, 1967). Ceruloplasmin is always turning over within the body, contributing to around 0.5mg of excreted copper per day (Sternlieb, 1967).

Copper homeostasis in the body is tightly regulated (Siotto & Squitti, 2018). Movement of copper throughout the body is facilitated by a variety of copper-containing proteins, some of which reduce copper^{+2} to copper^{+1}, which later reverts back to copper^{+2} (a.k.a. Cu(II)/Cu(I) redox cycling). Copper-containing proteins can be divided up into three classes (Siotto & Squitti, 2018):

- Cuproenzymes: proteins using copper as a cofactor, which act as chemical catalysts and undergo controlled Cu(II)/Cu(I) redox cycles, such as ceruloplasmin.
- Trafficking proteins: proteins that carry copper as "cargo" (copper trafficking) and do not undergo these controlled redox cycles, such as albumin and copper metallothionein
- Intrinsically disordered proteins (IDPs): flexible proteins without a defined structure, such as beta-amyloid—a protein linked to Alzheimer's disease (see *Focus Question #1*).

Trafficking proteins and IDPs have a lower affinity for copper than cuproenzymes. If copper increases in an unregulated way within these pools, it can end up producing hydroxyl radicals (ROS) that cause damage (Siotto & Squitti, 2018). In living organisms, copper doesn't statically exist in one pool or the other, but rather it exists in an equilibrium between different pools. It continually releases from tissues, exists as a free form, binds to small molecules, is incorporated into proteins, and so forth. Abnormalities in copper regulation have been linked to Alzheimers disease, diabetes, Parkinson's disease, acute inflammatory responses, and stroke (Siotto & Squitti, 2018).

One mechanism of copper dysregulation appears to relate to excess blood sugar, which can react with proteins such as albumin (Siotto & Squitti, 2018).[41] Albumin that reacts with sugar becomes "glycated," reducing its affinity for binding copper. These protein modifications also lead to the creation of "advanced glycation end products" (AGEs), also known as glycotoxins. Glycotoxins can modify blood vessels, creating new copper binding sites that trap copper. The copper can produce ROS in an unregulated manner, especially as albumin's ability to help maintain homeostasis is reduced under these conditions. This leads to a repeated cycle of stress that ultimately damages tissues (Siotto & Squitti, 2018).

Mutations in genes responsible for copper homeostasis can lead to inheritable copper toxicosis, notably Menkes syndrome and Wilson's disease (Harris & Gitlin, 1996). In Wilson's disease, the body is unable to excrete copper properly through the biliary system, and the body is unable to incorporate copper into newly synthesized ceruloplasmin. This dysfunction in homeostasis mechanisms causes copper concentrations to increase to toxic levels. In Menkes syndrome, copper builds up inside of cells early in fetal development. Dysfunction in copper transport in the body causes mental deterioration, hypothermia, connective tissue abnormalities, and death during early childhood. Both diseases are caused by defective copper transport proteins (Harris & Gitlin, 1996).

Health effects from exposure to elevated levels of copper

In a model organism (rats), copper hydroxide, copper oxychloride, Bordeaux mixture, tribasic copper sulfate and copper(I) oxide mixed with copper sulfate pentahydrate all exhibited similar absorption, distribution, and excretion rates (EFSA, 2018). The EPA notes that the cupric (copper 2^+) ion is the component of copper fungicides that is of "toxicological interest" (EPA, 2009). For simplicity, we are considering exposure to copper from different sources to behave similarly. It is worth noting, however, that at least one study has shown that other ingredients in copper products can affect their toxicity (See *Evaluation Question #7*). Additionally, EPA data shows that copper toxicity varies by both material and route of exposure, which is described below.

Humans are exposed to copper when breathing air, drinking water, ingesting food, and making skin contact with copper-containing substances (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). Exposure to high levels of copper (for example, due to occupation or intentional/accidental ingestion) can cause the following (Agency for Toxic Substances and Disease Registry (ATSDR), 2004; Richardson, 2000; Royer & Sharman, 2022):

- nose, mouth, eye, and skin irritation
- headaches, dizziness, coma, and altered thinking
- nausea, stomach cramps, vomiting, and diarrhea
- kidney damage
- liver failure
- red blood cell death, tachycardia, cardiac failure
- respiratory issues, such as mucous membrane damage, pulmonary fibrosis, abnormal masses, possibly cancer
- death (large doses, often taken intentionally to commit suicide)

The EPA concluded that acute exposure effects are due to the body's response to limit absorption of copper (EPA, 2009). While this may be, studies of toxicity in many other organisms show that copper poisoning can cause cellular damage through a variety of mechanisms, such as the production of reactive oxygen species (e.g., membrane damage) and through enzyme interference (see *Evaluation Question #5*). In other words, in most organisms, cells exposed to higher concentrations of copper can be damaged by it directly. This is supported as well by Prasad (2008), who notes that copper toxicity in humans is a result of oxidative damage to membranes and macromolecules. Royer & Sharman (2022) state that excess copper causes oxidative stress, DNA damage, and reduced cell proliferation—symptoms that relate to the direct effects of copper.

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[41] Albumin is an important protein, involved in a wide variety of functions within the body, including metal scavenging and acting as an antioxidant (Fanali et al., 2012).

When human cells are directly and chronically exposed to copper in the laboratory, two proteins are produced as a protective measure (Jiménez et al., 2002). These proteins are glutathione and metallothionein. While these proteins normally help prevent damage caused by excess concentrations of copper, metallothionein can become unstable when cells undergo additional stresses. Under these circumstances, metallothionein can switch from acting as an antioxidant, to an *oxidant* within the cell, causing damage. In a study with liver cells (HepG2), Jiménez et al. (2002) found that chronic exposure to excess (but "non-toxic") concentrations of copper caused a large increase to intracellular copper, as well as a large increase in metallothionein production. Additionally, the copper treatment decreased available glutathione, essentially binding all of the proteins and keeping them from offering further protection. When the cells were subjected to a peroxide source,[42] the treated cells were much more likely to undergo oxidative stress and die (lysis).

Toxicity quantification and exposure

The EPA considers copper products to generally have moderate (EPA Toxicity Category II) to low acute toxicity (Toxicity Category III-IV), depending on exposure route and the specific substance itself (EPA, 2009). Some copper products though have high acute toxicity (eye irritation; Toxicity Category I), specifically copper sulfate and copper hydroxide (see Table 14, below).

Table 14: Available Acute Toxicity Studies on Copper-Containing Compounds, adapted from U.S. EPA, 2009

Copper Type	Acute Oral LD_{50}(mg/kg)	Acute Dermal LD_{50}(mg/kg)	Acute Inhalation	Primary Eye Irritation	Dermal Irritation	Dermal Sensitization
Copper(II) sulfate pentahydrate (99%)	Male (M)=790 Female (F)=450 Tox Cat II	>2000 Tox Cat IV	None Available	Severe eye irritation day 1 to day 21 Tox Cat I	Non-irritating Tox Cat IV	None Available
Copper(II) sulfate basic	None Available					
Copper(II) hydroxide (77%)	M=2253 F=2160 Tox Cat III	>2000 Tox Cat III	M=1.53 mg/L F=1.04 mg/L Tox Cat III	Irritative Corneal opacity, iris irritation, chemosis, invasion of cornea by blood vessels Tox Cat I	At 72 hrs, very slight erythema Tox Cat IV	Non-sensitizing Guinea Pig
Copper(II) oxide (97.6%)	>5050 (M&F) Tox Cat IV	>2020 (M&F) Tox Cat III	>2.08 (M&F) Tox Cat III	Irritation cleared in 7 days Tox Cat III	Irritation cleared day 21 PI Index=1.49 Tox Cat III	Non-sensitizing (guinea pig)
Cuprous oxide (83.9%)	Tox Cat III	Tox Cat IV	Tox Cat IV	Tox Cat III	Tox Cat IV	Non-sensitizing (guinea pig)
Copper(II) oxychloride (94.1%)	M=1537 F=1370 Tox Cat III	M&F=710 Tox Cat II	>1.7 mg/L Tox Cat III	Corneal opacity redness and vascularization Tox Cat I	Non-irritating Tox Cat IV	Nonsensitizing
Copper(II) oxychloride sulfate	None Available					
Copper(II) ammonia complex	None Available					
Copper(II) ethylenediamine complex	M=527 F=462 Tox Cat II	>2000 Tox Cat III	>2000 Tox Cat III	Moderate irritation Tox Cat III	Redness, edema, cleared by day 3	non sensitizing guinea pig
Copper salts of fatty acids and rosin acids (Cu	> 7000 Tox Cat IV	> 2000 Tox Cat III	None Available	no irritation Tox Cat IV	Edema, erythema, PIS=1.0 Tox Cat III	None Available

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[42] Peroxides are biologically significant molecules, involved in a variety of cellular processes.

Copper Type	Acute Oral LD_{50}(mg/kg)	Acute Dermal LD_{50}(mg/kg)	Acute Inhalation	Primary Eye Irritation	Dermal Irritation	Dermal Sensitization
& zinc neoisoate 35%)						
Copper(II) octanoate, 10% fatty acids	>2000 M&F Tox Cat III	>2000 M&F Tox Cat III	>0.38 M&F Tox Cat III	Irritation cleared by 48 hrs. Tox Cat IV	slight erythema, edema, cleared by 72 hrs. Tox Cat IV	Non-sensitizing guinea pig

Prasad (2008) noted that atmospheric concentrations of copper normally range from 5 to 200 ng/m^3, but sometimes reach as high as 100 mg/m^3. Under those rare circumstances, it is possible that copper intake through inhalation could be as high as 2000 mg in a single 24 hour period (Prasad, 2008).

According to the EPA "based on available data, there is no evidence that warrants determining any dietary, oral, dermal or inhalation endpoints to quantify sub-chronic and chronic toxicity." In other words, the EPA in 2009 did not believe that copper products posed a risk from chronic occupational exposure, and they dismissed medical case studies because they didn't include the specific quantities of copper patients were exposed to. However, other sources indicate that chronic exposure to copper products may pose damaging health effects, especially to the respiratory system (Agency for Toxic Substances and Disease Registry (ATSDR), 2004; Jiménez et al., 2002; Šantić et al., 2005). Additionally, the CDC notes that copper pipes are a concerning source for copper contamination in drinking water (CDC, 2018).

Concerns over copper pipes and chronic copper ingestion have existed since at least the early 1970's (Salmon & Wright, 1971). In a case report, Salmon & Wright describe a 15-month-old infant admitted to a hospital with a 5-week history of behavioral changes, diarrhea, severe undernourishment, and other symptoms. The only identifiable cause was elevated copper levels in blood serum. After being treated with a chelating agent, the infant's condition improved. Salmon & Wright concluded that the cause of the elevated copper levels in the infant were due to a hot water system containing copper piping, which the family used regularly for cooking and drinking. Testing showed that the infant's home had much higher levels of copper in the water than the hospital and other areas in the city (Salmon & Wright, 1971).

In many cases, the lowest inhalation exposure levels causing symptoms in humans can't be determined because information comes from medical case reports (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). Some of these case reports include exposure levels to copper dust concentrations within the range of 111—434 mg/m^3 for a period of over three years. However, other substances are often present, which can potentially obscure causal agents. For example, while studies have shown that copper mine workers are at greater risk for stomach and lung cancer, copper ores also contain other materials such as silica and iron, which could not be ruled out as contributors (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). However, agricultural workers exposed to copper pesticides have also shown higher risks for cancer (Šantić et al., 2005).

In Europe, EFSA (2018) concluded that agricultural workers in vineyards and tomato fields were exposed to levels of copper fungicides that exceeded acceptable levels for safety. This is true even when recommended PPE is worn when re-entering tomato fields after treatment with copper fungicides. EFSA recommended that the acceptable operator exposure level should be 0.08 mg Cu/kg per day (EFSA, 2018).

According to EFSA (2018), "realistic" exposures to copper products does not produce evidence of immunotoxicity or disruption of the endocrine system in humans. Copper products are also not considered toxic by EFSA for reproduction or cancer (EFSA, 2018). EFSA identified that there is a possible link between copper and Alzheimer's disease, but found that the hypothesis was not substantiated. According to a review by Bost et al. (2016), copper intake between 0.6 to 3 mg/day does not pose a risk of cardiovascular disease, cognitive decline, arthritis or cancer.

Bordeaux mixture

One of the most common combinations of copper with another material is Bordeaux mixture (Alloway, 2013). This is a mixture of copper sulfate and calcium hydroxide [$Ca(OH)_2$] or quick lime (CaO) (Gowariker et al., 2008). Mixing the two ingredients forms copper(II) hydroxide, which is stabilized with calcium sulfate, also produced in the double-displacement reaction (Dixon, 2004). Use of Bordeaux mixture began in 1882, and has been used ever since to control a variety of diseases such as powdery mildew, potato late blight, scab and fire blight of apple, and citrus canker (Gowariker et al., 2008; Dixon, 2004).

Long-term exposure to Bordeaux mixture frequently causes chronic respiratory symptoms, including cough, chest constriction, and breathing difficulty (Šantić et al., 2005). This is often known as "vineyard sprayer's lung" or VSL. Researchers have noticed that animals and workers exposed to the substance form the following in the lungs (Šantić et al., 2005):

- small inflamed regions (granulomas)
- increased amounts of collagen
- connective tissue growing within what would normally be fluid-filled space in the lungs (interstitium)
- copper formations in the cytoplasm of immune cells (macrophages)
- lesions

Pimentel and Marques (1969) observed this condition in vineyard workers, and were able to reproduce it in guinea pigs over the course of five months. Several other studies have identified respiratory disease in wine and grape workers exposed to pesticides (Zuskin et al., 1997). According to the EFSA (2018), these cases were likely associated with onsite preparation of Bordeaux mixture and "application techniques at higher rates than those used in modern agriculture."

In a study of vineyard workers in Bosnia and Herzegovina, researchers found that non-smoking vineyard workers were roughly four times more likely to develop lung cancer than similar individuals in the area who did not work in vineyards, and who were not exposed to Bordeaux mixture on a regular basis (Šantić et al., 2005). A similar study of 174 vineyard and orchard workers found that those exposed to Bordeaux mixture were significantly more likely to have difficulty breathing (dyspnea), chest tightness, chronic cough, and chronic phlegm than workers employed as food packers (Zuskin et al., 1997). The EPA (2009) believed that the case for copper involvement in vineyard sprayers lung was "not definitive" because researchers did not identify possible exposures to other pesticides.

Studies in mice have shown that copper oxide nanoparticles (25 nm in diameter) can cause lung inflammatory responses after a single exposure to 3.6 mg/m^3 for four hours (Gosens et al., 2016). In rats, 50 nm copper oxide nanoparticles caused inflammation at doses of 0.17 and 0.5 mg/rat, when injected into the trachea. At 2 mg/rat, these tracheal treatments (to the lungs) led to death within 1 day of the exposure. In a different study, rats were exposed to copper oxide nanoparticles through the nose over a 5-day period. After that period, tissue damage in the animals was consistent with:

- alveolitis
- bronchiolitis
- an increase in vacuole formation
- emphysema
- epithelium degeneration in nasal passages.

These effects were seen beginning with 2.4 mg CuO/m^3 to the highest does given of 13.2 mg/m^3 (Gosens et al., 2016).

Evaluation Question #11: **Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).**

Due to its wide range of antimicrobial activity, its multiple modes of action, and its low cost, identifying single substance alternatives to copper is difficult and often controversial for crop producers (Tamm et al., 2022). There are no known alternatives to copper for treatment for some bacterial plant diseases, such as citrus canker. Other bacterial diseases in citrus, blueberry, apple, cherry, strawberry, pepper, and tomato may not have registered antibiotic treatments (EPA, 2009). Particularly in Europe, significant and urgent research is ongoing in an effort to find alternatives to copper pesticides (Andrivon et al., 2020; Dagostin et al., 2011; Tamm et al., 2022). European nations have recently been reducing the maximum allowable copper application rates due to its environmental impacts, and some countries have banned its use altogether, leading to uneven competition and fears of a complete continental prohibition (Andrivon et al., 2020).

Jamar & Lateur (2007) demonstrated that a mixture of wettable sulfur and lime sulfur was only slightly less effective than a copper and sulfur mixture in the prevention of apple scab on infection-susceptible Pinova apples. Potassium bicarbonate was somewhat effective, but less so than either aforementioned mixture. The effect could be improved with the usage of appropriate stickers and spreaders, since potassium bicarbonate is soluble and decomposes quickly (Jamar & Lateur, 2007). Elemental sulfur, lime sulfur, and potassium bicarbonate are permitted synthetic substances for plant disease control at 7 CFR §205.601(i). Other non-copper materials allowed at 205.601(i) are hydrogen peroxide, and oils, such as horticultural or vegetable oil. Many of these are commercially available for organic production (OMRI, 2022).

Cantonwine et al. (2008) compared the efficacy of a variety of fungicides permitted in organic crop production for the treatment of leaf spot in peanuts in field trials in the Southeastern United States (Georgia and North Carolina). The researchers found that sulfur treatments had moderate efficacy, but did not approach the success of copper(II) sulfate or copper(II) hydroxide treatments. Sulfur also induced phytotoxicity, so the authors advised against the use of sulfur in this pathosystem unless copper toxicity is already a concern. Neem oil was ineffective. Copper fungicide alone had the same fungal inhibition effect as copper mixed with *Bacillus subtilis*, and the authors did not include a trial for *Bacillus subtilis* alone (Cantonwine et al., 2008).

In comparative efficacy trials on potato late blight, Dorn et al. (2007) tested 53 copper-free compounds against copper fungicides, including plant extracts, microbial preparations, and mineral formulations. None of the compounds they tested approached the efficacy of copper to control the pathogen in field trials, though limited success was described for cultures of *Xenorhabdus bovienii*, *Trichoderma harzianum*, and *Bacillus subtilis* microbes, as well as an aluminum sulfate clay/yeast formulated product (Dorn et al., 2007). Aluminum sulfate clays appear to work by reducing pH on the plant surface (by formation of sulfuric acid from the sulfate anion) and the inhospitable spore germination environment created by aluminum (Zwieten et al., 2007). The stone meal, enzymatic preparations, and plant extracts tested either had no effect or even injured the plants (Dorn et al., 2007).

Few grapevine varieties resistant to downy mildew are available, limited mostly to Germany and Switzerland, but resistant options are gradually becoming more available (Pertot et al., 2017). The search for alternatives to copper for this pathosystem is important for the European wine industry (Dagostin et al., 2011). In controlled indoor trials, Dagostin et al. (2011) tested more than 100 treatments to control downy mildew in grapes, and identified 38 substances determined to be worthy of field trials when compared to traditional copper(II) hydroxide treatments. Of these compounds selected for field study, 6 copper-free substances prevented more than 60% of foliar infections, and 17 prevented more than 60% of infections on grape bunches (Dagostin et al., 2011). Copper(II) hydroxide prevented 68-100% and 61-99% on leaves and bunches, respectively (Dagostin et al., 2011). The most effective protection against downy mildew in grapevines occurred with (Dagostin et al., 2011):

- *Bacillus subtilis* bacteria (the EPA registered fungicide "Serenade")
- *Trichoderma harzianum* fungus
- potassium bicarbonate
- aluminum sulfate clay
- saponins from yucca
- Rhamnolipid biosurfactant (the EPA registered "Zonix Biofungicide" derived from fermentation of *Pseudomonas aeruginosa* bacteria)
- the fatty acid lauric acid
- thyme oil
- citrus oil
- sage extract

- *Inula viscosa (*a Mediterranean perennial herb) extract

Alternative substances approved in Europe and shown to have some efficacy against fungal disease include (Andrivon et al., 2020; Trebbi et al., 2021):

- potassium bicarbonate
- sodium bicarbonate
- calcium hydroxide
- sulfur (often combined with copper)
- horsetail extract
- nettle extract
- willowbark extract
- citrus oils

Other substances not yet approved in Europe (as of 2020) that show some promise are (Andrivon et al., 2020):

- steam distilled essential oils of thyme
- summer savory
- oregano
- clove
- tea tree and extracts of yucca
- five-seeded plume-poppy
- garlic
- Sodom apple (not a true apple)
- *Reynoutria sachalinensis* (knotweed)
- sage
- black poplar
- licorice
- chinaberry

However, tea tree oils have been shown to damage fragile fruit like blueberries, and *Reynoutria sachalinensis* has been shown to injure mites in the ecosystem (Zwieten et al., 2007).

Giant knotweed extract was sold in Europe starting about 1995 as the formulation Milsana. It is now commercially available in the U.S. as the formulation Regalia, which is approved for use in organic agriculture (OMRI, 2022). It is extremely effective for powdery mildew on grapes, tomatoes, and cucumber (Quarles, 2009).

Horsetail (*Equisetum arvense*) contains silica, palmitic acid, 3-deoxy-d-mannoic lactone, and dehydroxyacetone, and nettle (*Urtica dioica*) contains imidazoles (carbon nitrogen ring compounds) which have all shown antimicrobial or plant-strengthening activity (Langa-Lomba et al., 2021; Trebbi et al., 2021). When mixed with chitosan, extracts of these two plants have been shown to be particularly effective against fungal diseases occurring in the woody trunk of grapevine in Spain (Langa-Lomba et al., 2021). When used alone, the plant extracts exhibit a moderate inhibitory effect, implicating a synergistic effect related to chemical complexing with chitosan (Langa-Lomba et al., 2021). Horsetail extract has also been shown to be effective against late blight (*Phytophthora infestans*) in tomato, possibly equally as effective as copper(I) oxide, copper(II) hydroxide, and Bordeaux mixture for moderate to severe infection in Italian organic farms (Trebbi et al., 2021). Horsetail also has a moderate effect against brown rust (*Puccinia triticina)* in Durum wheat, but appears to fail in combating severe infection (Trebbi et al., 2021).

A silica-based alternative to horsetail is potassium silicate. This material has been successfully used to prevent powdery mildew on cucumber, and might be useful for other diseases (Bowen et al., 1992; Chérif et al., 1992; Quarles, 2019). Aqueous sprays of potassium silicate can reduce the number of powdery mildew colonies on grapes and squash (Bowen et al., 1992; Menzies et al., 1992). Potassium silicate is allowed for use in organic agriculture at 205.601(i)(1), and formulations are commercially available (Quarles, 2019).

Silica from horsetail and potassium silicate probably prevents disease through induced systemic resistance.[43] More information on induced systemic resistance can be found within the Chitosan technical report, beginning on line 389 (NOP, 2020).

Plant extracts, even when very effective, may have limited potential for vineyards on large scales due to cost, availability, lack of persistence, and lack of tenacity (Dagostin et al., 2011; Gessler et al., 2011).

While several biocidal plant extracts, microbial metabolites, or mineral formulations have some efficacy against *fungal* infections typically targeted with copper pesticides, the only substance reported by Andrivon (2020) to have use against *bacterial* infection is vinegar. Some research has described moderate success with the use of bacteriophages in the treatment of citrus canker, but disease prevention approaching that provided by copper was not observed, and further research is needed (Balogh et al., 2008; Ibrahim et al., 2017).

There are several microorganism-based biological control products on the market, many of them EPA registered for uses overlapping with copper pesticides (Andrivon et al., 2020). Several of them are commercially available for organic production (OMRI, 2022).

<u>Evaluation Question #12:</u> Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

Efforts are underway to reduce the use of copper fungicides in European organic agriculture, but a complete phase-out is expected to result in significant yield losses across numerous crops, notably grapes, olives, and almonds (Andrivon et al., 2020; Tamm et al., 2022). Copper fungicides are used on a wide variety of crop and pathogen systems, making it difficult to narrow down specific alternative practices appropriate for all regions and climates (Tamm et al., 2022).

In the case of orchard crops, removing leaf litter during the dormant phase, or shredding and burying leaf litter within rows seems to be an effective method for reducing the release of fungal spores (Andrivon et al., 2020). Simple removal of infected tissues or, in more extreme outbreaks, complete plant removal, are also effective control methods (Andrivon et al., 2020).

Andrivon et al. (2020) describes a variety of preventive cultural, biological, and mechanical methods that are typical hallmarks of disease prevention in organic crop systems:

- Targeted application of nitrogen-rich material to accelerate decomposition of leaf litter, reducing the leafy nutrient source for dormant pathogens
- Crop rotation of annual crops and no-till methods
- Cover cropping between primary crops
- Careful selection of disease free seeds and starts
- Protective coverings like rain/hail shelters, greenhouses, or cold frames; European trials showed 90-100% effectiveness when installing rain shelters to prevent apple scab
- Application of antagonistic fungal species (such as *Trichoderma harzianum* and *Macrosphaeropsis ochracea*), which compete for resources with pathogens
- Removal or isolation of crop waste
- Selective pruning to "train" plant architecture, reducing microclimates favorable to pathogen development
- Mixed species plots

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[43] Induced systemic resistance, or ISR, is a process in which a plant responds to the introduction of a biological organism or substance with enhanced immune system activity, leading to increased defense against infection by a wide spectrum of pathogens (Pieterse et al., 2014). Researchers in the past two decades see great promise in the exploitation of ISR in sustainable crop production (Choudhary et al., 2007; Pieterse et al., 2014).

The application of mature composts or compost tea has been shown to suppress the spread of diseases as well through a complex mechanism that includes increased microbial competition (Zwieten et al., 2007).

Tamm et al. (2022) conclude that the key factor for reducing copper fungicide usage in Europe is the wider introduction of disease resistant cultivars, but market preferences related to taste or appearance may preclude this. Producers may also choose to graft commercially preferred plants onto disease resistant rootstock (Zwieten et al., 2007). Andrivon et al. (2020) lists several heirloom apple, pear, and grape varieties resistant to infection in France, Canada, and the United States, and describes specific genes responsible for disease resistance in potato, tomato, and lettuce that may be exploited in the development of new cultivars. The use of rain shelters or other crop coverings to limit contact with air or waterborne spores may reduce reliance on copper fungicides, but also may be cost prohibitive (Andrivon et al., 2020; Tamm et al., 2022).

Mixing resistant and disease susceptible cultivars within rows may also help to alleviate reliance on copper pesticides by reducing pathogen reproduction and introducing physical barriers between plants (Andrivon et al., 2020). This mixing effect is only advantageous before pathogen populations are established, however (Andrivon et al., 2020).

Several biocontrol agents consisting of microbes, including biophages, are available that target pathogens sensitive to copper, but efficacy data outside of the laboratory is lacking (Andrivon et al., 2020). These microbial agents have overlap as applied substances, while also qualifying as biological control practices. Biological controls that have shown promise against fire blight include formulations containing the bacteria *Pseudomonas fluorescens*, *Pantoea agglomerans*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, the yeast *Aureobasidium pullulans*, and the antibiotic kasugamycin (NOP, 2021). Andrivon et al. (2020) states that adoption of resistant cultivars combined with the use of biocontrols might allow the complete abandonment of copper as a disease control substance, but significant research is needed.

Focus Questions Requested by NOSB Crops Subcommittee

Focus Question #1: Discuss whether there is scientific evidence that application of copper products for agricultural use contributes to Alzheimer's disease or other brain disorders (and summarize if information is available).

Considerable effort has been directed toward finding a link between Alzheimer's disease (AD) and copper exposure. According to Coelho et al. (2020) more than 3,000 papers have been published with the terms "Alzheimer's disease and copper." Plaque deposits in the brain, characteristic of Alzheimer's disease, have enriched levels of copper, zinc, and iron (Bagheri et al., 2018). This is well established in literature. However, the significance of these heavy metals in plaques, and whether copper dysregulation is directly involved, is more contentious. Bagheri et al. (2018) cite about 25 papers supporting loss of copper and zinc homeostasis as a possible contributing factor in Alzheimer's disease. But according to EFSA (2018), "A possible causative link between disturbed copper homeostasis and Alzheimer disease pathology in humans has been hypothesized but could not be substantiated." Taylor et al. (2020) reach similar conclusions. Part of the difficulty in establishing whether copper dysregulation could contribute to Alzheimer's is that there are still aspects of the disease that are not well understood. For example, it is possible that there are different forms of the disease. If copper dysregulation is involved with Alzheimer's disease pathology, it is likely a subset of patients (Siotto & Squitti, 2018).

Alzheimers disease is characterized by the presence of beta-amyloid protein clumps (plaques) between neurons in the brain (NIH, 2017). Accumulations of abnormal tau proteins form "tangles" within neurons as well, especially in later stages. These tangles disrupt neuron function. Beta-amyloid and tau appear to be interrelated in the progression of Alzheimer's disease. When the concentration of beta-amyloid clumps outside of neurons reaches a threshold, tau protein rapidly spreads throughout neurons in the brain. In healthy brains, glial cells help to clear away potentially damaging protein collections and other wastes, but in humans with Alzheimer's, glial cells fail to do this. Eventually, neurons are injured and die throughout the brain, ultimately leading to death (NIH, 2017).

As mentioned earlier, some studies suggest that copper homeostasis is compromised in humans with Alzheimer's disease (Atwood et al., 2000). A meta-analysis of studies on serum, plasma, and cerebrospinal fluids found that copper levels in the plasma of people affected by Alzheimer's disease was higher than in people that were healthy (Ventriglia et al., 2012). A separate meta-analysis found an increase in 18 studies, no difference in 14, and a decrease in one study (Bagheri et al., 2018). Meanwhile, a similar analysis indicated that there is an overall copper *decrease* within the brain[44] (Siotto & Squitti, 2018). If copper dysregulation is involved with Alzheimer's disease, it is not as simple as copper occurring in elevated levels—the organ of interest in this case actually has *lower* levels of copper, while outside the brain, the levels are higher. However, copper is still occurring at high levels within plaques in the brain. This pattern is similar to that in Wilson's disease: less copper bound to cuproenzymes, and more copper loosely bound to other substances (Siotto & Squitti, 2018). However, Wilson's disease patients do not generally show Alzheimer pathology (Siotto & Squitti, 2018).

Within the body, most copper is bound to the protein ceruloplasmin (Harris & Gitlin, 1996). The fraction of copper *not* bound to ceruloplasmin is considered the "exchangeable" copper within the body (see also *Evaluation Question #10 – Regulation of copper levels in humans*). This is the fraction believed to play a role in Alzheimer's disease, as well as diabetes (Siotto & Squitti, 2018). Of note, type II diabetes is a risk factor for Alzheimer's disease (Siotto & Squitti, 2018). People with higher-than-normal free copper within the body have a 10-times greater risk of Type 2 diabetes than similar people with normal free copper levels (Squitti et al., 2017). Similarly, higher-than-normal free copper is associated with a three-times greater risk of Alzheimer's disease in patients with Mild Cognitive Impairment (MCI) (Siotto & Squitti, 2018). Fifty to sixty percent of MCI and AD patients have copper abnormalities (Siotto & Squitti, 2018). One explanation for why only 50-60% have copper abnormalities is that copper-related Alzheimer's disease is a subtype.

Why do plaques have high levels of metals, such as copper? Beta-amyloid (Aß) proteins, which form plaques, come in different sizes (Atwood et al., 2000). The Aß1-40 size is the main form, and usually remains soluble in biological fluids. In contrast, Aß1-42 is the form found more commonly in plaque deposits. Copper^{2+} ions, as well as zinc and iron have been found to bind to histidine amino acid residues within beta-amyloid proteins. Complexes of beta-amyloid and copper precipitate, forming plaques as they do. Postmortem studies have shown that adding copper chelators to beta-amyloid plaques removes copper, allowing the proteins to become soluble again. Aß1-42 has a much stronger affinity for copper ions than Aß1-40. Aß1-42 forms aggregations of proteins as it precipitates, even when only very small amounts of copper are available. The solubility of both forms of beta-amyloid are pH dependent (Atwood et al., 2000).

Another hypothesis is that degeneration of neurons may not be the direct activity of plaques, but instead of soluble beta-amyloid fragments, bound to heavy metals such as copper, zinc, and iron (Streltsov et al., 2008). Twenty to forty percent of cognitively normal older people have brain plaques, and therefore plaques themselves may not be toxic (Kepp & Squitti, 2019). Instead, soluble beta-amyloid fragments are hypothesized to bind to Cu^{2+} and form complexes that are chemically reactive, facilitating the production of damaging ROS (Siotto & Squitti, 2018).

Yet another hypothesis for how copper dysregulation could contribute to Alzheimer's disease relates to its well-known ability to create ROS. As noted in *Evaluation Question* #10, research suggests that events leading to the production of glycotoxins can damage albumin and modify proteins in blood vessels (Squitti et al., 2017). These changes allow exchangeable copper to cycle between oxidation states, and in the process create damaging ROS— potentially contributing to both diabetes and Alzheimer's disease (Squitti et al., 2017).

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[44] However other studies indicate that copper accumulates in the brain as we age (Coelho et al., 2020). It could be that the decrease referred to within this study was related to non-exchangeable forms, namely ceruloplasmin.

Mutations in another copper trafficking protein, ATP7B, are involved in some cases of Alzheimer's disease, as well as Wilson's disease (Siotto & Squitti, 2018). Some researchers believe that Alzheimer's disease is likely not a single clinical entity, but rather a disease caused by different issues (Siotto & Squitti, 2018).

For copper-dysfunction related Alzheimer's disease, causes may be the following (Kepp & Squitti, 2019):

- an increase in ATP7B copper trafficking protein variants
- an increase in the proportion of exchangeable copper (not bound to ceruloplasmin)
- a decrease in the activity of ceruloplasmin
- a decrease in copper bound to proteins in the brain (higher proportion of free/exchangeable copper)

Work by Brewer (2010, 2012, 2017) has suggested that copper exposure could be a risk factor. According to Brewer:

- In the 19th century, Alzheimer's-like disease was rare in developed countries,[45] but increased to 15% in people aged 65-74, and 44% aged 75-84 during the 20th century.[46]
- This trend is not seen in undeveloped countries, where occurrence is approximately 1%.
- This trend appears to correspond to an increase in the ingestion of copper^{2+} from:
    - drinking water from copper pipes, used in developing countries
    - eating more meat, from which copper is absorbed at a 50% greater rate compared to non-meat sources.

While Brewer's hypothesis could be true, much of it is based on circumstantial evidence. Patterns in Alzheimer's disease around the globe do indicate that it is more prevalent among the elderly in some areas than others (Patel & Aschner, 2021). For example, the rate is 1.6% for people over 60 in Africa, while it is 4.0% in China, 4.6% in Latin America, 5.4% in Western Europe, and 6.4% in North America (Patel & Aschner, 2021). However, in contrast to Brewer, Patel & Aschner (2021) conclude that genetic, vascular, and psychosocial factors have stronger epidemiologic, neuroimaging, and neuropathological evidence for Alzheimer's disease compared with dietary, nutritional, or occupational exposure to toxic chemicals.

Brewer (2017) argues against genetics as a primary factor, because the incidence has increased over the last century. He believes that shouldn't be enough time to see such a dramatic increase in Alzheimer's disease incidence. Not all of the evidence that Brewer uses is entirely circumstantial, either. A 2003 study by Sparks & Schreurs looked at the effects of water sources, cholesterol, and copper on rabbits (Sparks & Schreurs, 2003). They found that adding small amounts of copper to distilled drinking water (0.12 ppm—approximately 1/10th that allowed by EPA in drinking water) for 10 weeks caused rabbits to accumulate beta-amyloid protein and brain plaques. However, as stated earlier, it is still unclear whether brain plaques themselves are the pathological cause of Alzheimer's disease.

In a review by Coelho et al. (2020), several animal studies were reported that showed adding copper to diets caused accumulations of beta-amyloid protein, neuron death, cognitive issues, oxidative stress in brains, and other symptoms. The animals studied included flies, mice, rats, rabbits, and dogs.

Additionally, they noted that the prevalence of Alzheimer's disease in China is 2.6 times higher in areas with high soil copper content (60-80 ppm), compared with areas with lower copper content (20-40 ppm). However, according to Fagnano et al. (2018), copper soil levels up to 217 ppm do not generally lead to large concentrations in plants and food. Phytotoxic problems often (though not always) occur in plants before

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[45] Alzheimer's disease was not named until 1907 (Brewer, 2017)

[46] These values differ from those found in the *2022 Alzheimer's Association Facts and Figures* report (Alzheimer's Association, 2022). According to this report, Alzheimer's disease in the United States affects :

- 10.7% of people aged 65 and older
- 5.0% of people aged 65-74
- 13.1% of people age 75-84
- 33.2% of people aged 85 and older

high levels can build in edible portions. A food survey in Italy found that the highest concentration observed (3.9 ppm Cu in peach, fresh weight) was 64% lower than that presenting a risk to human health (11 ppm) (Fagnano et al. 2018).

<u>Focus Question #2:</u> Discuss the prevalence of copper in animal manure, and manure's role in the accumulation of copper in the agro-ecosystem.

Copper is prevalent in animal manure, and manure is a large source of copper in the agro-ecosystem. Pig manure is estimated to contribute 50% of the copper to the environment in Denmark (Brandt et al., 2008), and livestock manure may account for 69% of the soil copper input in China (Niu et al., 2013). In Europe, livestock manures are the largest contributors of copper to soils, accounting for 29-72% of the total input (Alloway, 2013). We were unable to find studies that documented the amount of copper in manure in the United States and, therefore, its contribution to soil. For a discussion of the issues with copper accumulation, see *Evaluation Questions #5-9*.

Copper additives are used in animal feed to increase growth and prevent diseases (Xiong et al., 2010). Concentrations are used in feed far beyond what is necessary for nutrition (Alloway, 2013). For example, only 4-5 ppm Cu in food are required to meet nutritional requirements, but concentrations of up to 140 ppm were allowed in Europe in the past (Alloway, 2013). The excess copper is intended to act as a growth promoter. A recent review article recommended that 75 to 250 ppm Cu be given to pigs in order to improve feed intake (Espinosa & Stein, 2021).

Much of the excess copper fed to animals is excreted or moves through the gut unabsorbed, concentrating it in the manure (Xiong et al., 2010). When this manure is spread on land, copper accumulates—especially given its low mobility in soil (Xiong et al., 2010). In one study, Xiong et al. (2010) collected samples of both animal feed and animal manure around Beijing and Fuxin, China. They found that copper was concentrated in animal manure, compared to what they were fed in their diet (see Table 15, below). Feed values for pig, cattle, and chicken were similar to those found on farms in England and Wales. The authors inventoried copper inputs to soils in various counties in China, and found that pig manure was the primary contributor in almost all counties except one, where sheep manure was the largest source of copper. The loading rates due to manure were substantial. They estimated that depending on the county, it would take 13-95 years to raise the soil from 35 ppm to 100 ppm (Xiong et al., 2010).

Table 15: Concentrations of copper in animal feed and animal manure (mg/kg or ppm) in China. Adapted from Xiong et al., 2010.

Source	Value type	Pig	Cattle	Chicken	Sheep
Feed	Range	2.2-395.2	0.3-141.4	6.7-175.9	0.7-34.8
	Mean	131.7	22.4	29	11.3
Manure	Range	50-2016.7	5.6-112.9	22.15-477.5	14.9-510.5
	Mean	699.6	31.8	81.9	66.9

Manure on its own can increase the bioavailability of copper as well, by forming complexes with DOM. A study by Brandt et al. (2008) used bacteria that luminesce when exposed to bioavailable copper to investigate the effect of pig manure on soil. The researchers found that pig manure had the effect of dramatically increasing the bioavailability of copper, while counterintuitively reducing free copper^{2+} ion in the soil. This study is significant, because in addition to the effect of manure, it shows that free copper^{2+} ions are not necessarily always a good measure of bioavailability. Copper complexes with dissolved organic matter were found to be bioavailable to the *Pseudomonas sp.* used in this experiment (Brandt et al., 2008). Likewise, In Saskatchewan, researchers demonstrated that application of cattle and pig manure fertilizers significantly increased the bioavailability of copper in wheat and barley (Lipoth & Schoenau, 2007).

Report Authorship

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Appendices

Appendix A: Chemical and Physical Properties of Relevant Copper Compounds

copper(II) sulfate (anhydrous): $CuSO_4$

Property	Value[a]
Other Names	Cupric sulfate
CAS	7758-98-7
Other Codes	EPA PC Code: 024408; FDA UNII: KUW2Q3U1W
Physical State and Appearance	Crystalline or powder
Color	White or off-white
Molecular Weight (g/mol)	159.61
Percent Copper	39.8
Density (g/cm^3)	3.6
Solubility (g/100ml, in water, 20 °C)	20.3
Boiling Point	650 °C
Melting Point	590 °C
Stability	Hygroscopic, decomposes upon heating
Reactivity	Reacts with metals and some organic solvents

[a] **Source:** (National Center for Biotechnology Information, 2022d)

copper(II) sulfate pentahydrate: $CuSO_4 \cdot 5H_2O$

Property	Value[a]
Other Names	Cupric sulfate; bluestone; blue vitriol; chalcanthite
CAS	7758-99-8
Other Codes	EPA PC Code: 024401; FDA UNII: LRX7AJ16DT
Physical State and Appearance	Crystalline or powder
Color	Blue to ultramarine
Molecular Weight (g/mol)	249.69
Percent Copper	25.4
Density (g/cm^3)	2.3
pH	3.5-4.5
Solubility (g/100ml, in water, 20 °C)	32
Boiling Point	653 °C (decomposes)
Melting Point	110 °C (decomposes)
Stability	Stability indefinite when dry[b]
Reactivity	Corrosive to steel

[a] **Source:** (National Center for Biotechnology Information, 2022e)

[b] Copper(II) sulfate pentahydrate dehydrates with increasing temperature. At 88-100 °C, trihydrate first forms. At 114 °C, monohydrate forms. At 240-245 °C, anhydrous copper(II) sulfate forms.

copper sulfate basic: $3Cu(OH)_2 \cdot CuSO_4$

Property	Value[a]
Other Names	Tribasic copper sulfate; brochantite,
CAS	1344-73-6
Other Codes	EPA PC Code: 008101
Physical State and Appearance	Crystalline or Powder
Color	Light blue-green
Molecular Weight (g/mol)	452.29
Percent Copper	56.2
Solubility (g/100ml, in water, 20 °C)	Nearly insoluble
Boiling Point	Decomposes above 380 °C
Melting Point	Decomposes above 380 °C
Stability	Stable
Reactivity	Nonvolatile

[a] **Source:** (National Pesticide Information Center, 2012; Richardson, 2000)

copper(II) hydroxide: $Cu(OH)_2$

Property	Value[a]
Other Names	Cupric hydroxide; copper dihydroxide
CAS	20427-59-2
Other Codes	EPA PC Code: 023401; FDA UNII: 3314XO9W9A
Physical State and Appearance	Crystalline or powder
Color	Blue-green
Molecular Weight (g/mol)	97.56
Percent Copper	65.1
Density (g/cm^3)	3.37
Solubility (g/100ml, in water, 20 °C)	Nearly insoluble
Boiling Point	n/a; decomposes to copper oxide
Melting Point	80 °C
Stability	Decomposes to copper oxide
Reactivity	Complexes with ammonia

[a] **Source:** (National Center for Biotechnology Information, 2022g)

copper(II) oxide: CuO

Property	Value[a]
Other Names	Cupric oxide; copper oxide black; tenorite
CAS	1317-38-0, 1344-70-3
Other Codes	EPA PC Code: 042401; FDA UNII: V1XJQ704R4
Physical State and Appearance	Crystalline, powder, or granules
Color	Black
Molecular Weight (g/mol)	79.55
Percent Copper	79.9
Density (g/cm^3)	6.315
Solubility (g/100ml, in water, 20 °C)	Practically insoluble
Boiling Point	Decomposes to copper(I) oxide
Melting Point	1326 °C
Stability	Stable
Reactivity	Reduced by hydrogen peroxide, ammonia, and carbon monoxide

[a] **Source:** (National Center for Biotechnology Information, 2022j, 2022c; Patnaik, 2003)

copper(I) oxide: Cu_2O

Property		Value[a]
Other Names		Cuprous oxide; Copper oxide red; dicopper oxide; cuprite
CAS		1317-39-1
Other Codes		EPA PC Code: 025601; FDA UNII: T8BEA5064F
Physical State and Appearance		Crystalline or powder
Color		Red-brown
Molecular Weight (g/mol)		143.09
Percent Copper		88.8
Density (g/cm^3)		6.0
Solubility (g/100ml, in water, 20 °C)		Insoluble
Boiling Point		1800 °C
Melting Point		1235 °C
Stability		Stable in dry conditions
Reactivity		Oxidizes readily, corrosive to aluminum

[a]**Source:** (National Center for Biotechnology Information, 2022a)

copper(II) oxychloride: $Cu_2Cl(OH)_3$

Property	Value[a]
Other Names	Dicopper chloride trihydroxide; basic copper chloride
CAS	1332-65-6
Other Codes	EPA PC Code: 023501; FDA UNII: 76712031PG
Physical State and Appearance	Crystalline or powder
Color	Green-blue
Molecular Weight (g/mol)	213.57
Percent Copper	59.5
Density (g/cm^3)	3.77
Solubility (g/100ml, in water, 20 °C)	Practically insoluble
Boiling Point	Decomposes before boiling
Melting Point	Decomposes before melting
Stability	Decomposes on heating to copper oxide and HCl
Reactivity	Corrosive to iron and other metals

[a]**Source:** (Committee for Risk Assessment, 2014; National Center for Biotechnology Information, 2022k)

copper(II) carbonate hydroxide: $Cu_2CO_3(OH)_2$

Property	Value[a]
Other Names	Basic copper carbonate; cupric carbonate; malachite
CAS	12069-69-1
Other Codes	EPA PC Code: 022901; FDA UNII: GIK928GH0Y
Physical State and Appearance	Crystalline or powder
Color	Green-blue
Molecular Weight (g/mol)	221.12
Percent Copper	57.5
Density (g/cm^3)	3.76
Solubility (g/100ml, in water, 20 °C)	0.00012
Boiling Point	Decomposes before boiling
Melting Point	Decomposes before melting (200 °C)
Stability	Decomposes on heating
Reactivity	Nonvolatile

[a]**Source:** ([EC] European Communities, 2011)

copper ammonia complex: $Cu(NH_3)_4^{2+}$

Property	Value[a]
Other Names	Tetraamminecopper(II) ion[b]; copper ammine complex
CAS	16828-95-8
Other Codes	EPA PC Code: 022702; FDA UNII: J68H5PUV30
Physical State and Appearance	n/a[c]
Color	Blue
Molecular Weight (g/mol)	131.67
Percent Copper	48.3
Density (g/cm^3)	n/a[c]
Solubility (g/100ml, in water, 20 °C)	n/a[c]
Boiling Point	n/a[c]
Melting Point	n/a[c]
Stability	n/a[c]
Reactivity	n/a[c]

[a]**Source:** (National Center for Biotechnology Information, 2022h)

[b]There are several varieties of copper ammonia complex, differing by the number of ammonia ligands attached to the copper constituent (Hathaway & Tomlinson, 1970). The CAS number appearing on the EPA exempt list is the tetraammine complex containing 4 ammonia ligands.

[c]As an ion, the tetramminecopper complex can bond to countless other single or polyatomic ions and exhibit several stereoisomers, so precise chemical and physical property data will vary (Hathaway & Tomlinson, 1970).

Copper ethylenediamine complex: $C_4H_{14}CuN_4$

Property	Value[a]
Other Names	Cupriethylenediamine; copper 2-azanidylethylazanide
CAS	13426-91-0
Other Codes	EPA PC Code: 024407; FDA UNII: NIP4I4LVCC
Physical State and Appearance	Liquid
Color	Purple
Molecular Weight (g/mol)	181.73
Percent Copper	35.0
Density (g/cm^3)	Depends on concentration in solution
Solubility (g/100ml, in water, 20 °C)	n/a[b]
Boiling Point	n/a[b]
Melting Point	n/a[b]
Stability	Stable
Reactivity	Dissolves cotton and wood, and corrosive to metals

[a]**Source:** (National Center for Biotechnology Information, 2022f)

[b]The concepts of solubility, boiling point, and melting point of metal complexes is difficult to describe because these properties rely on the starting metal salt reactant and are affected by the occurrence of other organic or inorganic ligands in a medium. In general, complexing metals increases the solubility of the starting material (Tapparo et al., 2020).

copper octanoate: $C_{16}H_{30}CuO_4$

Property	Value[a]
Other Names	Copper caprylate; copper soap; octanoic acid, copper salt
CAS	20543-04-8
Other Codes	EPA PC Code: 023306
Physical State and Appearance	Liquid
Color	Light blue
Molecular Weight (g/mol)	349.95
Percent Copper	18.2
Density (g/cm^3)	1.048 (depends on concentration)
Solubility (g/100ml, in water, 20 °C)	Low solubility, but dispersable in water
Boiling Point	Depends on concentration
Melting Point	Depends on concentration
Stability	Stable
Reactivity	Incompatible with oxidizers and acids

[a]**Source:** (EPA, 1997b; National Center for Biotechnology Information, 2022i)

Appendix B

Use Rates for EPA registered Copper Products, as measured by cupric ion content. Adapted from US EPA, 2009

Copper Refined Actual Use Rates for Crops			
Crop	**Maximum per Application Rate (lbs Cu^{2+}/A)[1]**	**Maximum Annual Rate (lbs Cu^{2+}/A)[2]**	**Minimum Retreatment Interval [3]**
TREE FRUIT			

Copper Refined Actual Use Rates for Crops				
Pome Fruit (apple, loquat, pear, quince	Fall, late dormant	8.0	16.0	n/a (only 1 application per season permitted)
	Between silver-tip and green-tip	6.0		n/a (only 1 application per season permitted)
	Bloom, growing season	1.5		5 days
Atemoya, Sugar Apple		3.15	12.6	7 days
Avocado		3.15	18.9	14 days
Banana		1.05	18.9	7 days
Carambola		2.1	10.5	7 days
Cherimoya (custard apple)		2.1	8.4	14 days
Citrus (citron, grapefruit, kumquat, lemon, orange, pummelo, tangelo, tangerine, lime		3.15	12.6	7 days
Guava		1.23	4.92	7 days
Mamey Sapote		2.1	8.4	14 days
Mango		3.2	48.0	7 days
Olive		6.0	18.0	30 days
Papaya		2.63	21.2	7 days
Persimmon		1.0	6.0	14 days
Stone Fruit (apricot, cherry, nectarine, peach, plum, prune)	Dormant, late dormant, up to pink bud	8.0	18.0	7 days
	Bloom/growing season	1.5		5 days
TREE NUTS				
Almond	Dormant, late dormant	8.0	18.0	7 days
	Bloom/growing season	1.5		5 days
Betel Nut (Guam)		0.75	8.25	7 days
Cacao		2.25	15.75	14 days
Chestnut		2.1	8.4	14 days
Coffee		2.1	12.6	14 days
Filbert[1]		6.0	24	14 days
Litchi		1.23	4.92	7 days
Macadamia		2.36	9.44	7 days
Nutmeg		2.1	8.4	14 days
Pecan, Pistachio		2.1	8.4	14 days
Walnut		4.0	32.0	7 days
FIELD CROPS				
Alfalfa		0.53	1.12	30 days
Cereal Grains (barley, millet, oat, rye, sorghum, wheat)		0.53	1.06	10 days
Clover		0.53	4.74	7 days
Corn (Field Corn, Popcorn, Sweet Corn)		1.05	4.2	7 days
Peanut		0.79	4.74	7 days
Potato		2.5	25	5 days
Soybean		0.79	4.74	7 days
Sugar Beet		1.31	7.86	10 days
Sugarcane		0.53	1.06	10 days

Copper Refined Actual Use Rates for Crops			
Tobacco	2.0	8.0	10 days
SMALL FRUITS			
Artichoke	0.53	2.65	7 days
Asparagus	1.0	5.0	10 days
Bean (Dry, Green)	0.79	4.74	7 days
Beet (Table Beet, Beet Greens)	1.31	7.86	10 days
Carrot	1.0	5.0	7 days
Celery, Celeriac	1.0	5.3	7 days
Chard	0.79	3.95	7 days
Crucifers (broccoli, Brussels sprout, cabbage, cauliflower, Chinese cabbage, collard greens, kale, kohlrabi, mustard greens, turnip greens)	0.53	2.65	7 days
Curcubits (cantaloupe, casaba, chayote, citron melon, cucumber, gourd, honeydew, muskmelon, pumpkin, squash (summer and winter), watermelon, waxgourd)	1.05	5.25	5 days
Eggplant	0.79	7.9	7 days
Garlic	1.0	6.0	7 days
Leek	1.0	6.0	7 days
Lettuce (endive, escarole)	1.0	8.0	5 days
Okra	1.05	5.25	5 days
Onion	1.0	6.0	7 days
Pea	0.79	3.95	7 days
Pepper (bell, chili)	0.79	11.85	3 days
Radish	1.31	7.86	10 days
Rhubarb	0.79	3.95	7 days
Rutabaga	1.31	7.86	10 days
Shallot	1.0	6.0	7 days
Spinach	0.79	3.95	7 days
Tomato (processing)	0.53	17.4	3 days
Tomato (fresh market)	1.6	8.0	3 days
Turnip	1.31	7.86	10 days
Watercress	0.53	2.12	7 days
VINES			
Grape	3.0	20.0	3 days
Hops	0.53	2.65	10 days
Kiwi	2.1	6.3	30 days
Passion Fruit	2.36	9.44	7 days
MISCELLANEOUS			
Chicory	1.31	7.86	10 days
Chives	0.53	2.65	7 days
Cinnamon	3.15	18.9	14 days
Coriander	0.53	2.65	10 days
Dill	0.79	3.95	7 days
Ginseng	1.05	5.25	7 days
Mint	0.53	2.65	10 days
Parsley	1.0	2.0	10 days
Rosemary	0.53	2.65	10 days
Turfgrass	3.0	21.0	10 days

Copper Refined Actual Use Rates for Crops			
ORNAMENTALS			
[2]Lilies, Easter	2.5	75.0	7 days
All Other Ornamentals	2.0	20.0	7 days

1 Permitted only in Washington State and Oregon

2 Maximum pounds of metallic copper which may be applied to an acre for each application. Product labels must also include application rates described in liquid units or pounds of total product.