Environmental Toxicology and Chemistry—Volume 43, Number 1—pp. 19–30, 2024
Received: 5 April 2023 | Revised: 15 May 2023 | Accepted: 16 October 2023

Critical Perspectives

Further Limitations of Synthetic Fungicide Use and Expansion of Organic Agriculture in Europe Will Increase the Environmental and Health Risks of Chemical Crop Protection Caused by Copper-Containing Fungicides

Quentin C. Burandt,[a,b] Holger B. Deising,[c] and Andreas von Tiedemann[a,*]

[a]Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Georg-August-University Göttingen, Göttingen, Germany
[b]Institute of Plant Breeding, Seed Science and Population Genetics, Division of Crop Biodiversity and Breeding Informatics, University of Hohenheim, Stuttgart, Germany
[c]Institute of Agricultural and Nutritional Sciences, Division of Phytopathology and Crop Protection; Martin Luther University Halle-Wittenberg, Halle, Germany

Abstract: Copper-containing fungicides have been used in agriculture since 1885. The divalent copper ion is a nonbiodegradable multisite inhibitor that has a strictly protective, nonsystemic effect on plants. Copper-containing plant protection products currently approved in Germany contain copper oxychloride, copper hydroxide, and tribasic copper sulfate. Copper is primarily used to control oomycete pathogens in grapevine, hop, potato, and fungal diseases in fruit production. In the environment, copper is highly persistent and toxic to nontarget organisms. The latter applies for terrestric and aquatic organisms such as earthworms, insects, birds, fish, *Daphnia*, and algae. Hence, copper fungicides are currently classified in the European Union as candidates for substitution. Pertinently, copper also exhibits significant mammalian toxicity (median lethal dose oral = 300–2500 mg/kg body wt in rats). To date, organic production still profoundly relies on the use of copper fungicides. Attempts to reduce doses of copper applications and the search for copper substitutes have not been successful. Copper compounds compared with modern synthetic fungicides with similar areas of use display significantly higher risks for honey bees (3- to 20-fold), beneficial insects (6- to 2000-fold), birds (2- to 13-fold), and mammals (up to 17-fold). These data contradict current views that crop protection in organic farming is associated with lower environmental or health risks. Further limitations in the range and use of modern single-site fungicides may force conventional production to fill the gaps with copper fungicides to counteract fungicide resistance. In contrast to the European Union Green Deal goals, the intended expansion of organic farming in Europe would further enhance the use of copper fungicides and hence increase the overall risks of chemical crop protection in Europe. *Environ Toxicol Chem* 2024;43:19–30. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Copper; Earthworms; Ecotoxicology; Fungicide; Mammalian toxicology; Soil contamination; Pesticide risk assessment; Crop protection

INTRODUCTION

Copper is a heavy metal and a transition element. As an essential micronutrient, it is indispensable for numerous metabolic functions in almost all organisms (Kaim & Rall, 1996; Pascaly et al., 1999). Copper compounds are also among the longest-used active ingredients in plant protection (Beye, 1974; Schmitt, 1969; Schwab, 2000). As early as 1720, copper sulfate was used as a fungicide, initially as a cereal seed dressing (Schwab, 2000). In 1807, Benedict Prévost discovered that treating grains with copper salts reduced the development of wheat blight (Ayres, 2004). Toward the end of the 19th century, copper sulfate was used to control weeds in viticulture (Schmitt, 1969), and at the beginning of the 20th century, copper served as a herbicide in cereals (Schwab, 2000).

Importantly, in the early 1880s, the French botanist Pierre-Marie Alexis Millardet discovered the fungicidal effect of copper against downy mildew of grapevine (Ayres, 2004). After Millardet's experiments with different concentrations of lime

---

* Address correspondence to atiedem@gwdg.de

DOI: 10.1002/etc.5766

and copper sulfate, the mixture became the first commercially successful fungicide and was named "Bordeaux broth" in 1885 (Ayres, 2004; Clark, 1902). The effectiveness of "Bordeaux broth" also against other pathogens and in other crops subsequently made copper the most abundantly used fungicide in crop protection (Clark, 1902). Until well into the 20th century, application rates of 20 to 30 kg of copper per hectare were common, and sometimes up to 80 kg per hectare and year were applied (Kühne et al., 2009). The improvement of copper formulations and the discovery of dithiocarbamates, the first organic synthetic fungicides, as well as forecasting models and breeding of tolerant crop cultivars has contributed to a reduction in the historically high amounts of copper applied (Kühne et al., 2009).

The approval and use of copper as fungicide in crop protection to date is essentially based on its indispensability in organic farming. Nevertheless, because of its critical toxicological and ecotoxicological properties, a withdrawal of the active ingredient approval is being considered by the European Commission (Tamm et al., 2021), which has led to the assignment of copper fungicides as candidates of substitution. In this context, the present review aims to (1) provide an overview of the mode of action and uses of copper in crop protection, (2) highlight the risks associated with copper fungicides in terms of phytotoxicity, mammalian toxicity, and ecotoxicity, and (3) critically discuss the current status of efforts to reduce copper application. Because the evaluation, approval, and use of copper-containing fungicides in crop protection are regulated at an European Union level, our analysis with a focus on Germany can be considered relevant for all European Union member states.

Mode of action

Copper compounds belong to the group of contact fungicides; uptake through the plant cuticle hardly occurs (Berger et al., 2012). In contrast to plants, fungal spores can take up copper ions as well as complex-bound copper. As a transition element, copper can easily interconvert between Cu^{1+} and Cu^{2+}, making this heavy metal an ideal redox component in copper-containing proteins such as cytochrome c oxidase, superoxide dismutase, ascorbate oxidase, microbial tyrosinases, and polyphenol oxidases called laccases, which are required for full virulence in almost all microbial pathogens (Rolke et al., 2004; Siafakas et al., 2006; von Tiedemann, 1997; Zhu et al., 2001).

Copper's cytotoxic effects have primarily been related to free metal ions (Knauert & Knauer, 2008; Morel et al., 1978; Sunda & Guillard, 1976). Importantly, unlike the mode of action of modern selective fungicides, the toxicity of copper cannot be related to one specific mode of action (Solioz, 2018). Copper binds to sulfhydryl groups, which determine protein structure and are thus indispensable for most, if not all, enzymes (De Filippis & Pallaghy, 1994; La Torre et al., 2018). Because of the enormous number of putative binding sites, it is plausible that copper affects several biochemical and developmental traits, resulting not only in a strong fungicidal but also a general biocidal effect (Fernandes & Henriques, 1991).

Liang and Zhou (2007) analyzed the toxic effect copper has on the model yeast *Saccharomyces cerevisiae*. These authors demonstrated that copper is capable of inducing extensive formation of reactive oxygen species (ROS) and apoptosis-like cell death. Apoptosis was indicated by cleavage of chromosomal DNA in copper-treated yeast cells (Madeo et al., 1997). Apoptotic DNA cleavage produces fragments with free 3′-OH termini, as detected by labeling with fluorescence-tagged nucleotides, a reaction catalyzed by an enzyme called terminal deoxynucleotidyl transferase (TUNEL assay). The TUNEL assay revealed intensive fluorescent nuclear staining in yeast cells already after 12 h of exposure to a copper concentration as low as 6 mM (Liang & Zhou, 2007).

In addition to apoptosis, ROS are generated in copper-treated yeast cells (see above). Because copper toxicity can be reduced by overexpression of superoxide dismutase, ROS appear to be functionally linked to copper toxicity. Because copper ions may block the glutathione reserves of the cell due to their high affinity for thiol groups, further stabilization and even an increase in the toxic effect may occur (Gisi, 2013) by compromising the ROS scavenging capacity of the cell. Visualizing the complexity of cellular stress responses to copper, microarray analyses indicated that in $CuSO_4$-treated yeast cells 143 open reading frames belonging to several distinct categories, including metabolism, cell cycle and DNA processing, transcription and translation, were induced more than twofold (Yasokawa et al., 2008). These data highlight the extent of biochemical de-regulation occurring in copper-treated fungi and contribute to understanding copper toxicity at the molecular and cellular level. Because the majority of these modes of action target highly conserved cellular functions, copper damages essential processes in bacteria, fungi, and mammals, making it a fairly toxic and nonselective plant protectant. This striking lack of selectivity provides a further strong argument for a phasing out of copper fungicides.

Forms of application

Besides the "Bordeaux broth" ($[Cu(OH)_2]_X \times CaSO_4$), copper has also been used in the form of various copper oxides and chlorides. In Germany, only plant protection products containing the active substances copper oxychloride ($CuCl_2 \times 3Cu(OH)_2$), copper hydroxide ($Cu(OH)_2$), and tribasic copper sulfate ($CuSO_4 \times 3Cu(OH)_2$) are currently approved (Table 1; Bundesamt für Verbraucherschutz und Lebensmittelsicherheit [BVL], 2022a). Compared with the original "Bordeaux broth," the more recent chemical forms allow a more efficient distribution of copper on the plant surface, which contributes to a reduction of applied copper dose rates.

Crops, target pathogens, and the use of copper-containing fungicides

The main area of use of copper-containing plant protection products is the control of oomycetes. Primarily based on their filamentous growth, oomycetes have traditionally been

TABLE 1: Copper-containing plant protection products authorized in Germany (BVL, 2022a)

Trade name	Approved until	Active substance	Pure copper content	Main crops (examples)	Target pathogens (examples)
Airone SC	31.03.2023	Copper oxychloride, Copper hydroxide	272.3 g/L	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
				Apple, pear, quince	*Erwinia amylovora*
BADGE WG	31.03.2023	Copper oxychloride, Copper hydroxide	279.97 g/kg	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
				Ornamental plants	*Puccinia allii*
COPRANTOL DUO	31.03.2023	Copper oxychloride, Copper hydroxide	279.97 g/kg	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
				Peach, plum	*Taphrina deformans*
Grifon SC	31.03.2023	Copper oxychloride, Copper hydroxide	272.3 g/L	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
				Ornamental plants	*Cercospora species*
Cuprozin progress	30.09.2023	Copper hydroxide	249.33 g/L	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
FUNGURAN-OH 50 WP	31.12.2026	Copper hydroxide	499.77 g/kg	Potato	*Phytophthora infestans*
Funguran progress	30.09.2023	Copper hydroxide	349.59 g/kg	Potato	*Phytophthora infestans*
				Grapevine	*Plasmopara viticola*
				Hops	*Pseudoperonospora humuli*
				Pome fruit	*Nectria galligena*
COBOX	31.12.2026	Copper oxychloride	499.92 g/kg	Potato	*Phytophthora infestans*
Flowbrix	31.12.2022	Copper oxychloride	379.61 g/L	Tomato, aubergine	*Phytophthora infestans*
				cucumber, courgette	*Pseudoperonospora cubensis*
Cuproxat	31.10.2023	Copper sulfate, tribasic	190.1 g/L	Grapevine	*Plasmopara viticola*

Pure copper contents are given as g/L or g/kg of the product. Pure Copper content is calculated from the Copper salt content in the plant protection product and the Copper content of the chemical formulation.

classified in the kingdom Fungi. However, modern molecular taxonomy suggests that oomycetes are more closely related to heterokont algae within the Stramenopiles and have little taxonomic relatedness to filamentous fungi (Kamoun, 2003). Of particular importance is the control of downy mildew on grapevine (*Plasmopara viticola*), downy mildew on hops (*Pseudoperonospora humuli*), and the causative agent of late blight or brown rot in potato and tomato (*Phytophthora infestans*). In fruit, vegetable, and ornamental plant cultivation, copper is also used to combat various fungal pathogens, primarily ascomycetes. In addition, some agents have a side effect against bacteria such as fire blight (*Erwinia amylovora*; BVL, 2022a; Kühne et al., 2017).

The Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL; German Federal Office of Consumer Protection and Food Safety) currently lists (as of October 23, 2022) 10 approved copper-containing plant protection products (Table 1). Four products combine copper oxychloride and copper hydroxide (Airone SC, BADGE WG, COPRANTOL DUO, Grifon SC). Three products only contain copper hydroxide (Cuprozin progress, FUNGURAN-OH 50 WP, Funguran progress), two contain copper oxychloride (COBOX, Flowbrix) and one (Cuproxat) contains tribasic copper sulfate. Because the authorization for Cueva expired on January 31, 2020, no plant protection products containing copper octanoate are currently available in Germany (BVL, 2022a, 2022b). The authorizations of most of the copper-containing plant protection products expire in the course of 2023. FUNGURAN-OH 50 WP and COBOX are authorized until the end of 2026.

In accordance with the German Plant Protection Act, manufacturers, first distributors, and importers must submit the quantity of copper-containing plant protection products annually marketed in Germany to the BVL. These numbers allow the rough estimation of the volume of copper-containing plant protection products applied in Germany, as well as the long-term trends (BVL, 2022c). After copper sales decreased in Germany from 2000 to 2012, quantities of pure copper compounds marketed steadily increased again from 2013 to 2021 and have remained stable on an elevated level since. In 2021, sales figures were as high as in 2003 (Figure 1). Intriguingly, in 2021, 383.95 t of copper hydroxide, 18.61 t of copper oxychloride, and 25.66 t of tribasic copper sulfate were sold, corresponding to a total of 275.16 t of pure copper at present annually applied in Germany. Clearly, these figures show that copper minimization strategies have not been successful, and copper application rates have not been significantly reduced in the last 10 years. Instead, the latest trend indicates an increase in copper applications.

Copper is used not only in organic farming, but also in conventional farming. It is important to note that conventional

FIGURE 1: Development of sales of copper-containing plant protection products in Germany from 2000 to 2021. Data from BVL (BVL, 2022c).

agriculture uses copper to prevent the evolution of fungicide-resistant pathogen populations (Wilbois et al., 2009). Because more single-site fungicides will be banned by stricter regulation, the use of multisite copper fungicides may therefore be expected to increase in the future to compensate for the narrowed range of modes of action (Oliveira-Garcia et al., 2021). Interestingly, in 2013, the amount of copper applied in conventional agriculture in Germany was estimated at 84.8 t of copper compounds, which corresponds to 76% of the total copper applied in crop protection, with the remaining 24%, that is, 26.5 t of copper, being applied in organic cultivation. However, copper in organic farming is used on a much smaller area of cultivation. In conventional cultivation, in 2013, copper was applied on an area of 75 200 ha, compared with 13 784 ha in organic cultivation. Thus, the average dose of copper applied per hectare was 1.9 kg in organic and 1.1 kg in conventional production. Such differences are much higher in viticulture, where 2.29 kg per hectare was applied by organic growers, as compared with 0.8 kg per hectare in conventional vineyards (Kühne et al., 2016). The total use of copper-containing fungicides in organic farming in 12 European countries was estimated at 3258 t in 2017. The amount of copper used was highly correlated to the organically managed area (Tamm et al., 2021).

State of approval of copper compounds as active substances in the European Union

Current approval of copper as active substance in fungicides in the European Union has been in place since January 2019 and will expire on December 31, 2025. In the Renewal Report of November 27, 2018, the European Union member states represented in the Standing Committee on Plants, Animals, Food and Feed agreed on a renewed and extended approval of copper-containing fungicides (Regulation (EU) 2018/1981 of 13 December 2018). With this regulation, copper compounds were classified as candidates for substitution (CFS) for the first time within a re-approval process. In the European Union, active compounds are labelled CFS when fulfilling at least two of the three persistent, bioaccumulative, toxic (PBT) cut-off criteria. Of these, copper indeed fulfills two, namely persistent and toxic. The approval is therefore only valid for a period of 7 years and as long as an adequate substitute has not been found. Because European Union member states are in charge of authorizing plant protection products, copper fungicides—in spite of copper being approved as active ingredients on a European Union level—are not authorized as plant protection products in Denmark, Sweden, Finland, the Netherlands, and Estonia.

Until the end of 2025, to protect nontarget organisms, applications are limited to a total of 28 kg of copper per hectare over 7 years, representing an average application rate of 4 kg of copper per hectare and year. Farmers can thus vary the amount of copper annually applied based on the requirements by the particular disease severities (European Union Commission, 2018).

Articles 24(1a) and (3b) of the European Union Organic Farming Regulation (EC) No 2018/848 of May 30, 2018, permit the use of active substances of mineral origin for plant protection. Thus, copper can be legally used in organic farming. The use of copper compounds in organic agriculture is further regulated in the European Union by the implementing Regulation (EU) 2021/1165 of July 15, 2021.

Many organic growers' associations impose more restricted quantity limits of copper use on their members. Bioland, a major German certification agency, allows the application of a maximum of 3 kg of copper per hectare and year, with an exception for hops, where 4 kg per hectare and year are accepted. The application is mainly limited to horticulture and perennial crops. Exceptions are possible for potatoes. If the application of copper is indispensable, the soil of treated fields must be continuously tested for its copper content (Bioland, 2022). Demeter, another German agency, only allows the use of copper in perennial crops. Averaged over 5 years, a maximum of 3 kg of copper per hectare and year may be applied, preferably in doses of less than 500 g of copper per spray (Demeter, 2022). Since 2009, some European Union member states, including Germany, have set the quantity limit to 3 kg of copper per hectare and year (4 kg of copper per hectare and year for hops), in accordance with the rules of the growers' associations (Kühne et al., 2016).

Hazards and risks associated with copper use

The hazard properties associated with copper use in crop protection have obtained increasing attention recently (Lamichhane et al., 2018) and have led legislators to classify copper compounds as CFSs in the current approval (see above). As a result, withdrawal and replacement of copper as active ingredient in crop protection products has become an issue. The main reason is the accumulation of copper in the soil and its ecotoxic effects as a heavy metal (Ballabio et al., 2018). Moreover, copper is relatively toxic to plants and mammals (La Torre et al., 2018). Toxicity to nontarget organisms is based on the same unspecific cell toxicity in target pathogens. Protein functions are disrupted due to the high affinity of copper for amino and carboxyl groups, and to its pronounced affinity for thiol groups, leading to loss of function of sulfur-containing proteins (see above).

Phytotoxicity

For almost any living organism, including plants, copper is an essential micronutrient (Andresen et al., 2018; Panagos et al., 2018). As a component of plastocyanin, for example, copper has an essential function in the thylacoid electron transport chain and thus in photosynthesis. Copper is also an essential co-factor of plant copper/Zn superoxide dismutases, which have important functions as ROS scavenging enzymes localized in the cytoplasm, the stroma of chloroplasts, and peroxisomes. Sufficient copper supply is therefore necessary for plants, and plant protection measures using copper can counteract deficiency. For example, the annual copper uptake of hops may reach up to 2 kg per hectare, a demand for copper that is relatively high compared with other micronutrients (Wilbois et al., 2009).

However, copper may be harmful to plants in two ways: as spray damage and through soil contamination. Due to the unselective action of copper, excessive use of copper-containing plant protection products is more likely to cause damage to plant tissues. The chemotherapeutic index of copper fungicides, which is the ratio between effective and phytotoxic doses, is rather unfavorable. Thus, careful and precise dosing of copper compounds is mandatory. Particularly at low temperatures and under humid weather conditions, damage to plants may occur. Typical symptoms are "russeting" of fruits, leaf necrosis, partial shedding, and reduced growth (Winkler et al., 2022; Figure 2). In hops, under warm and humid weather conditions, an excess of copper can lead to the development of light spots on the leaf blade, and the plant tissue may dry out (Calderwood et al., 2015).

Sensitive culltivars of fruit species, for example grapes, and ornamental plants are particularly at risk. For hops and apple, it is known that varieties differ with regard to tolerance to copper-containing plant protection products (Calderwood et al., 2015; LTZ, 2022). Further improvement of formulation may allow more efficient distribution of copper on the plant surface and thus a reduction in the required amount of copper and phytotoxicity risks. The latest development of an active ingredient, copper octanoate, displaying the lowest chemical copper load, is currently not approved as an active ingredient at European Union level.

Copper uptake by plants and soil organisms depends on the concentration of bioavailable copper in soil (Felgentreu et al., 2017). This proportion varies mainly depending on the total copper content and decreases with increasing soil retention time (Felgentreu et al., 2017), the chemical state and binding form, and soil conditions. Copper mainly occurs as a cation in soil but may be complexed in various ways such as with clay minerals, organic matter, phosphorus, and sulfate (Mackie et al., 2012). This chemical status determines mobility and thus bioavailability and leaching/translocation of copper in the soil (Berger et al., 2012; Mackie et al., 2012). As soil pH and cation exchange capacity decrease, mobility and thus the proportion of bioavailable copper increase (Berger et al., 2012; Felgentreu et al., 2017; Smolders et al., 2009). Conversely, bioavailability of copper decreases with increasing soil organic matter content (Sauve et al., 2000) as stable complexes of copper with low-molecular weight organic acids as well as with humic and fulvic acids are formed (Berger et al., 2012). Consequently, copper deficiency, for example leading to plant disorders called "heather bog disease," predominantly occurs in crops grown on organic soils such as peat. Interestingly, phytotoxic effects were also recorded in wheat grown on soils from former vineyards contaminated by long-term use of elevated copper doses (Michaud et al., 2007).

Mammalian toxicity

Copper is an essential micronutrient not only for plants and most microorganisms, but also for humans (Andresen et al., 2018; Panagos et al., 2018). For example, copper plays an important role in the formation of haemoglobin and haemocyanin (Solomon, 2009). Humans take up copper primarily through plant and animal products, drinking water and food

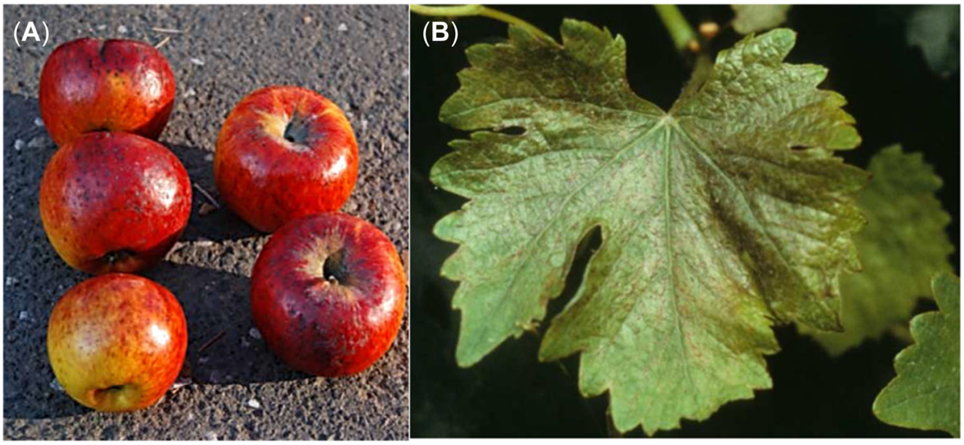

FIGURE 2: Phytotoxic effects of copper containing fungicides. (**A**) Russeting and necrosis on apple fruits. (**B**) Phytotoxic effects of copper on grapevine leaves treated under cool and humid conditions. Photographs: T. Röhmer, Versuchsstation für Obst- und Weinbau, Haidegg, Austria (left) and INRA, Ministry of Agriculture and Food, France (right).

supplements. Of minor importance is the intake via food supplements or biocides, such as some disinfectants (Michalski, 2015). The ingested copper is absorbed especially by the small intestine and in small amounts by the stomach. Intriguingly, the higher the copper intake, the lower the absorption (Itter & Pabel, 2013). After absorption, copper is passed on to the liver. Bound to caeruloplasmin, copper ions are distributed in the body through blood circulation (European Food Safety Authority [EFSA], 2018).

Humans and other mammals require a copper concentration of 5 to 20 µg per gram of body weight. Excess copper is secreted via kidneys and liver, and thus is regarded as harmless. These organs produce metallothionein, a protein that is able to bind copper. A water-soluble metallothionein–copper complex is formed that is excreted via the bile or faeces (Itter & Pabel, 2013; Solomon, 2009). However, the acute toxicity of copper-containing fungicides, as measured as median lethal dose (LD_{50}) in rats after oral uptake, is clearly higher than that of modern fungicides such as strobilurins (e.g., Kresoxim-methyl), and copper fungicides are also much more toxic than the herbicide glyphosate or even the neurotoxic pyrethroid insecticide Fenvalerate. For comparison of the toxicity of copper fungicides with that of other natural and synthetic compounds, see Figure 3. Importantly, copper fungicides are only moderately less toxic than DDT, which was banned in the 1970s because of its significant persistence in the environment and accumulation in food chains (see below). Although the mobility and environmental fate of copper and DDT are different, it must be emphasized that copper cannot be degraded and is even more persistent than DDT.

If the body's regulatory detoxification measures are not sufficient, symptoms of copper toxicity may occur. In rats, chronic oral copper sulfate exposure results in liver and kidney dysfunction as well as in neurobehavioral abnormality. In these organs, increased copper concentration was observed. In these studies, the liver was the most susceptible organ and copper toxicity increased in a dose- and duration-dependent manner of exposure (Kumar et al., 2015). Older findings suggest that the lungs may also be damaged. The term "vineyard sprayer's lung" refers to a disease occurring among vineyard workers characterized by detrimental changes in the lungs and liver. The disease is caused by exposure to "Bordeaux broth" over a long time in combination with old application techniques. The effect of the "Bordeaux broth" may have been exacerbated by the workers' tobacco consumption (EFSA, 2018).

In 2018, the EFSA found no evidence of direct adverse effects of copper on reproduction and fertility in rats. In mice, however, there were negative effects on development, with reduced weight of fetuses, increased fetal mortality, and malformations occurring at increased frequencies. Negative effects on development, to a lesser extent, have also been observed in rabbits. The German Federal Institute for Risk Assessment (BfR) also reported on weight and tissue changes in reproductive organs in animal experiments at doses as high as 12 mg/kg body weight (Itter & Pabel, 2013). Intriguingly, copper accumulation in the human brain may occur in patients suffering from heritable diseases such as Wilson's disease and Menkes syndrome. However, no neurotoxic effects have been reported for healthy people (EFSA, 2018).

There are different findings regarding the genotoxicity of copper. According to the EFSA, some DNA damage was observed in in vivo tests. Under normal conditions of use, however, this is unlikely to occur. Moreover, experiments on rats have not provided evidence for carcinogenic effects of copper nor have immunotoxic or endocrine disrupting effects been detected so far under realistic copper concentrations (EFSA, 2018).

In 2018, the EFSA confirmed the acceptable daily intake (ADI) for copper at 0.15 mg copper per kg body weight and day, which had already been defined by the European Union in 2008 (EFSA, 2018). The World health Organization had already set this value in 1996. Instead of a value of 0.2 mg copper per kg body weight and day, a lower value was used for infants

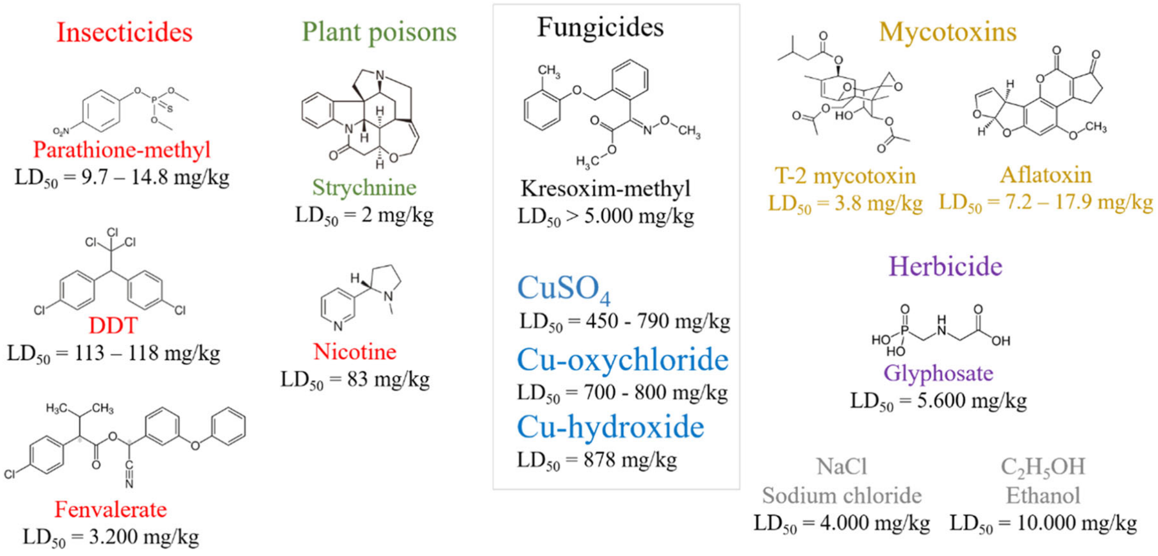

FIGURE 3: Median lethal dose (LD_{50}) values of different natural and synthetic compounds, as measured in rats after oral application. From Deising (2022) modified.

(World Health Organization, 1996). The BfR also emphasized an increased risk for children (Michalski, 2015). The acceptable operator exposure level, previously set at 0.072 mg copper per kg body weight and day, was raised by the EFSA to 0.08 mg copper per kg body weight and day (EFSA, 2018). The LD_{50} of copper is 115 µM in cell cultures (Singh et al., 2006) and 300 to 2500 mg/kg body weight (oral) in mammalians (European Chemicals Agency [ECHA], 2021). The mammalian toxicity of copper oxychloride and copper hydroxide is 10 to 20-fold higher than that of several modern synthetic fungicides with similar areas of use (Table 2).

Despite the broad range of general risks caused by increased copper exposure, various authors suggest that the risk of copper to consumers should not be overestimated in relation to its use in crop protection (Diesner et al., 2014; Wilbois et al., 2009). Phytotoxicity already occurs at copper concentrations that are not toxic to humans, and one may argue that this toxicity gap between plants and humans may represent an effective barrier for copper intoxication through ingestion with treated plant products (Wilbois et al., 2009). This is consistent with consumer exposure not exceeding 15.1% of the ADI in drinking water and 72.3% of the ADI in plant- and animal-based food (EFSA, 2018).

Ecotoxicity

Ecotoxicity and high persistence are the main reasons for the recurring discussion on withdrawing the approval of copper compounds in plant protection. Comparison of key ecotoxicological risk indices of the two most often used copper compounds indicates that copper hydroxide has a higher toxicity to aquatic organisms and a lower toxicity to terrestric organisms than copper oxychloride. Both copper compounds, however, are significantly more toxic to nontarget organisms than modern synthetic fungicides that are authorized for similar uses in crop protection, such as mandipropamid, zoxamide, cymoxanil, and fluopicolide (Table 2). This particularly concerns biological risks for terrestric organisms such as earthworms, honey bees, beneficials (parasitic wasps, predatory mites), birds, and mammals. Most strikingly, significantly increased ecotoxicity of copper compounds compared with modern synthetic chemistries is recorded for honey bees (3- to 20-fold), beneficial insects (6- to 2000-fold), birds (2- to 13-fold), and mammals (up to 17-fold). Modern synthetic chemistries also display significantly lower risks toward aquatic organisms compared with copper hydroxide, while copper oxychloride is in the same range. Boscalid, a compound used as a fungicide against ascomycetes and basidiomycetes, has a consistently more benign ecotoxicological profile than the two copper compounds. Besides the significantly more critical ecotoxicological data, the maximum total doses at which copper fungicides are applied, for example for the control of grapevine downy mildew, are four to 20 times higher than for modern synthetic fungicides (Table 2).

Soil contamination with copper can vary greatly depending on the crop, the year, and the region. During 2009 to 2014,

TABLE 2: Sensitivity of aquatic and terrestric organisms to copper containing active ingredients used in plant protection products and permitted maximum doses compared with modern synthetic fungicides

Target organism, parameter	Copper oxychloride	Copper hydroxide	Boscalid	Mandipropamid	Zoxamide	Cymoxanil	Fluopicolide
Aquatic							
Fish, acute 96 h, LC_{50} (mg/L)	>43.8	0.017	2.7	>2.9	0.16	29	0.36
Daphnia, acute 48 h, EC_{50} (mg/L)	0.29	0.038	5.3	7.1	>0.78	27	>1.8
Algae, acute 72 h, EC_{50} (mg/L)	165.9	0.009	3.8	>19.8	0.011	0.254	0.029
Terrestric							
Earthworms, acute 14 days, LC_{50} (mg/kg)	>490	>677	>500	>500	>1070	>1000	>500
Earthworms, chronic NOEC (mg/kg)	<40.5	<15	1.197	>16.0	66.7	6.6	62.5
Honey bee, oral acute LD_{50} (µg/bee)	12.1	49.0	>166	>200	>147	>85.3	>241
Beneficial insects, parasitic wasps, mortality, LR_{50} (g/ha)	3.97	50	>3600	827	>300	>480	8230
Beneficials, predatory mites mortality, LR_{50} (g/ha)	14.9	>149	>3600	>900	>300	>480	7130
Birds, acute, LD_{50} (mg/kg)	173	223	>2000	>1000	>2000	>486	>2250
Mammals, acute oral, LD_{50} rat (mg/kg body wt)	299	>489	>5000	>5000	>5000	356	>5000
Maximum permitted dose per application (g a.i./ha)	538[a]	613	600	120	180	90	100
Maximum total dose per season (g a.i./ha)	2688[a]	4290	600	360	360	270	200

[a] Badge contains 235.3 g/kg copper oxychloride and 215 g/kg copper hydroxide, calculation is based on copper oxychloride only, but the real copper dose is nearly double.

Dose comparisons are made for control of grapevine downy mildew in Germany.

Data sources: Pesticide Properties DataBase, University of Hertfordshire, UK, 2023; Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, online database, 2023. Doses data were used from solo formulations as far as available (copper hydroxide, Boscalid) and the doses shown are those permitted in grapevine for control of downy mildew (Boscalid: Botrytis grey mold). Products considered were copper oxychloride (Badge, Gowan Crop Protection Limited), copper hydroxide (Cuprozin Progress, Cosaco GmbH), boscalid (Cantus, BASF SE), mandipropamid (Ampexio, Syngenta Agro), zoxamide (Zorvec Vinabel, Corteva Agriscience Germany), cymoxanil (Afrasa Triple, Industrias Afrasa S.A.), fluopicolide (Profiler, BayerCropScience Germany).

LC_{50} = median lethal concentration; LD_{50} = median lethal dose; EC_{50} = median effect concentration; NOEC = no observed effect concentration; LR_{50} = median lethal rate (kg per hectare).

total copper loads between 7 and 97 mg per kg of soil were found in German orchards and 14 to 252 mg of copper per kg of soil in German vineyards (Felgentreu et al., 2017). While copper contamination in most fruit-growing areas (83%) in Germany can be classified as harmless, vineyards suffer much more from copper toxicity in the soil. A global survey on copper contents in vineyard soils revealed levels of contamination of up to 1500 mg/kg of total copper and 418 mg/kg of extractable copper (Mackie et al., 2012). A similar situation exists across Europe. While copper levels in arable soils are at 18.9 mg/kg on average, average levels in vineyard soils are 49.3 mg/kg, in orchards are 27.4 mg/kg, and in olive plantations are 33.5 mg/kg. Almost 15% of samples from European vineyards exceeded the critical value of 100 mg/kg (Ballabio et al., 2018). Interestingly, the total copper content did not vary significantly between organic and conventional farm soils. This is due to a long history of copper-intensive farming in all agricultural areas before synthetic fungicides became available, which has led to a significant background accumulation of copper (Felgentreu et al., 2017). However, because accumulation continues with further use, the present levels do not justify continued application of copper fungicides at any rates.

The bioavailability of copper for soil microbes is variable and depends on various factors, as described above for plant uptake. Copper damages various soil organisms after extended application. Among these, soil bacteria and soil fungi are particularly threatened because of the high bactericidal and fungicidal potential of copper (Bünemann et al., 2006). Consequently, soil microbial communities were significantly altered in copper polluted soils (Collins et al., 2012). Long-term use of copper fungicides in an avocado orchard resulted in reduced microbial biomass carbon and enhanced soil respiration (Merrington et al., 2002). Such effects may affect the essential soil functions required for the degradation of xenobiotics (Gaw et al., 2003). Copper may also amplify the antimicrobial effects of xenobiotics in soil. Copper enhanced the effects of sulfomethoxale, a sulfonamide contained in poultry farm manure, on soil microbial biomass, composition, and enzymatic functions. Significantly negative interactive effects were found on soil enzyme activities and the total soil microbial biomass. In addition, soil microbial composition was shifted by a significant decrease in the bacteria to fungi ratio (Liu et al., 2016). A recent report indicated another particular side effect of the extensive use of copper in orchards and vineyards resulting in the enhanced development of antibiotic resistance in *Escherichia coli* against chloramphenicol and tetracycline. The present study revealed that copper hydroxide fungicides induce upregulation of multidrug efflux pump genes and oxidative stress-related genes, which partially explains the emergence and selection of antibiotic resistance (Yu et al., 2022).

Under laboratory conditions, damage to soil invertebrates was observed at and above 55 mg of copper per kg of soil (Jänsch & Römbke, 2009). Among soil invertebrates, collembola are particularly sensitive to copper pollution (Frampton et al., 2006). Field studies with microarthropods indicated that the species composition of communities reacts more sensitively than the number of individuals within a single species population (Bruus Pedersen et al., 1999). Although a clear correlation between increased copper levels and decreasing abundance has only been found for sensitive species, the Shannon–Wiener index as a measure of biodiversity decreased linearly with increasing soil copper concentrations. This can be explained by the fact that individual species respond differently, leading to shifting of the species composition accordingly (Riepert, 2009). However, soil functions do not necessarily seem to suffer. For example, copper tolerance acquired on heavily polluted soils had no influence on the function of nitrifying microorganisms (Mertens et al., 2010).

Earthworms are among the most sensitive soil invertebrates to copper toxicity. Negative effects were observed in the field at and above 50 mg copper per kg soil (Jänsch & Römbke, 2009). This was confirmed by a study on growth of the earthworm *Eisenia fetida*, which was significantly reduced at and above 50 mg copper per kg soil (Zhou et al., 2013). Accordingly, the endpoints of no observed effect and lowest observed effect concentrations for copper toxicity to *Lumbricus rubellus* have been set to 10 and 40 mg/kg for metabolic responses, respectively (Bundy et al., 2008). In contrast to plants, earthworms also incorporate bound copper (Berger et al., 2012). Both the abundance and biomass of earthworms decreased with increasing copper input. In addition, higher species diversity among earthworms has been found in farming systems with low copper use, such as kiwifruit cultivation (Paoletti et al., 1998). Depending on their lifestyle, different earthworm species respond differently to increased copper doses. Results from previous studies indicate that endogeic earthworms inhabiting the topsoil are particularly affected by applications of copper compounds and elevated copper levels in soil (Felgentreu et al., 2017; Paoletti et al., 1998). Accordingly, anoecious earthworm species are less affected by soil pollution with copper (Felgentreu et al., 2017; Paoletti et al., 1998). Anoecious earthworms bore vertically through the soil and are thus only temporarily exposed to elevated copper concentrations in top layers. This implies that negative effects mainly derive from more recent copper applications, but not from copper already fixed in soil (Felgentreu et al., 2017). Copper contamination can vary greatly locally with the kind of cultivated crop. Because earthworms avoid substrates with higher copper loads, earthworm populations may recover in less polluted niches in the soil (Felgentreu et al., 2017).

Through surface leaching, copper can reach surface waters and be harmful to aquatic organisms. As with terrestrial organisms, both the bioavailability and concentration of copper are crucial for toxicity in water (de Oliveira-Filho et al., 2004). Elevated copper concentrations pose a high risk to fish and crustaceans. According to an earlier source (Solomon, 2009), these animals are 10 to 100 times more sensitive than mammals, and recent toxicity data suggest that these values even underestimate sensitivities (Table 2). In fish, as in other aquatic organisms, copper can accumulate in tissues (Padrilah et al., 2018). In addition to possible exposure through the food chain, aquatic animals are particularly threatened by copper through their gills, therefore irritated gills may be a symptom of increased copper exposure (Solomon, 2009). The exchange of salts such as sodium chloride and potassium chloride is disturbed, which can cause loss/disturbance of the salt balance of the body. Under normal conditions, these salts perform important functions in the cardiovascular and nervous systems (Solomon, 2009). Copper can also affect the sense of smell in fish by occupying binding sites on olfactory receptors and even causing their destruction in the long term. In addition, because the sense of smell significantly influences the behavior of fish, copper-induced behavioral disorders such as problems in salmon migration may occur (Baldwin et al., 2003). Other negative effects on reproduction, for example on egg and sperm production, have also been observed in various fish species (Solomon, 2009). Algae are even more affected by elevated copper concentrations than fish and crustaceans, which may render them less available as food in the event of copper contamination (Solomon, 2009). Significantly more information regarding the aquatic toxicity of copper would exceed the space limitation of this review. For details, the reader is referred to the US Environmental Protection Agency's water quality criterion document for copper (United States Environmental Protection Agency, 2007).

DISCUSSION

The present study summarizes key reports and documents to highlight the toxicological and ecotoxicological risks associated with the use of copper compounds in crop protection. Copper-containing fungicides are essential for organic farming, a growing agricultural sector in Europe that invariably depends on their use in crop protection (Tamm et al., 2021). Yield losses in organic production have been estimated at 50% to 100% if copper fungicides were no longer available as plant protection products in hops, grapes, and various fruit crops. For potatoes, losses have been estimated at 15% to 20%, while in vegetable and ornamental plant production losses of 10% to 15% would occur (Wilbois et al., 2009). More recent studies indicate that this dependency is continuing and that the unavailability of copper fungicides would render organic cultivation no longer profitable (Gitzel & Kühne, 2016; Kühne et al., 2017; Tamm et al., 2021). According to the current European Union regulation, copper may still be approved as an active substance because it fulfils only two of the three PBT criteria (European Union Commission, 2018). However, since 2015, copper has been listed as a candidate for substitution (ECHA, 2020; European Union Commission, 2018).

So far all efforts to develop formulations with lower copper contents have been unsuccessful and thus have not led to a significant reduction in copper application in agriculture. Similarly, the search for alternatives to copper fungicides permitted in the protection of organic crops has not been successful. The quantities permitted and used above all strongly depend on the disease pressure due to seasonal weather conditions and recent years have demonstrated that these rates may not be sufficient to protect susceptible crops. The current approval also takes into account that an active substance can be approved if it is of public interest and no sufficient substitute is available (ECHA, 2020). However, in contrast to the current goals of the European Green Deal, the societal as well as the political interest to maintain and even further expand organic farming will lead to an increase in copper use and thus worsen the ecological situation in European crop production.

In Germany, substantial funding, for example by the Federal Programme for Organic Farming and Other Forms of Sustainable Agriculture, provided by the Federal Ministry of Food and Agriculture, has promoted the search for alternatives allowing the replacement and minimization of copper from as early as 2001 (Gitzel & Kühne, 2016). Improved formulations and alternative chemical forms of copper have brought some progress, but have not sufficiently reduced copper loads. Similarly, minimization strategies including nonchemical measures of integrated crop protection, such as the introduction of new, more robust varieties, forecasting systems, and the promotion of advisory services, have not been sufficient to avoid severe crop damage at high disease pressure.

The development of new plant protection products based on natural substances such as plant extracts or microorganisms that can be used in organic farming may be a long-term alternative but currently cannot replace copper fungicides. Moreover, consumer risk associated with the use of natural compounds or antagonistic microorganisms remains to be carefully assessed (Deising et al., 2017; Oliveira-Garcia et al., 2021). Potential alternative agents such as potassium phosphonate, which has a significantly more favorable ecotoxicological profile than copper, had to be abandoned because the active ingredient was classified as a synthetic plant protection product and thus has not been permitted in organic farming since October 2013.

With its high persistence and toxicity to aquatic and terrestric nontarget organisms, copper compounds stand out compared with the modern synthetic fungicides currently approved in the European Union because they fall significantly below the high standards met by modern compounds. In addition, a recent EFSA statement pointed out that nondegradable transition metals such as copper used as active compounds in fungicides defy a proper environmental risk assessment because their behavior and toxicity are distinctive characteristics not covered in the current guidance documents for synthetic plant protection products. Because current risk assessment covers only a defined period of time, assessment of the long-term hazards of copper accumulated in particular environmental compartments is limited. As a result, the nondegradability of copper makes a robust estimation of long-term risks in the environment very difficult, if not impossible, and represents a consistent unresolved issue, although the protection goals for the environment remain the same as for synthetic plant protection products (EFSA PPR panel; Hernandez-Jerez et al., 2021).

Our comparison of copper fungicides with modern synthetics with regard to risks for nontarget organisms in agro-ecosystems indicates that their replacement by the latter would be a significant contribution to risk reduction in chemical crop protection in Europe. However, because important sectors in organic production essentially rely on the availability of copper compounds (Andrivon et al., 2018; Gitzel & Kühne, 2016) and substitutes meeting the organic certification requirements are not in sight, the future use of copper compounds in crop protection will clearly depend on the organic production area. Further expansion of organic farming, which is on the Green Deal agenda of the European Commission, will therefore inevitably further increase the use of copper compounds and result in significant worsening of the ecological quality of crop protection. European Union policies concurrently leading to a reduction of available synthetic fungicides will further aggravate this trend because copper fungicides will partially return to conventional production to fill the gaps and mitigate development of fungicide resistance caused by narrowing the range of available single-site synthetics.

Acknowledgments—We thank M. Streloke, Federal Office of Consumer Protection and Food Safety (BVL), for critical discussions and the three anonymous reviewers for many helpful suggestions. Open Access funding enabled and organized by Projekt DEAL.

Author Contributions Statement—**Andreas von Tiedemann**: Conceptualization; Supervision; Validation; Writing—review & editing. **Holger B. Deising**: Conceptualization; Visualization; Writing—review & editing. **Quentin C. Burandt**: Visualization; Writing—original draft.

Data Availability Statement—Not eligible.

REFERENCES

Andresen, E., Peiter, E., & Küpper, H. (2018). Trace metal metabolism in plants. *Journal of Experimental Botany*, *69*(5), 909–954.

Andrivon, D., Bardin, M., Bertrand, C., Brun, L., Daire, X., Fabre, F., Gary, C., Montarry, J., Nicot, P., Reignault, P., Tamm, L., & Savini, I. (2018). Can organic agriculture give up copper as a crop protection product? Condensed report of the Scientific Collective Assessment, INRA, 66p.

Ayres, P. (2004). Alexis Millardet: France's forgotten mycologist. *Mycologist*, *18*(1), 23–26.

Baldwin, D. H., Sandahl, J. F., Labenia, J. S., & Scholz, N. L. (2003). Sublethal effects of copper on coho salmon: Impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environmental Toxicology and Chemistry*, *22*(10), 2266–2274.

Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P., & Montanarella, L. (2018). Copper distribution in European topsoils: An assessment based on LUCAS soil survey. *Science of the Total Environment*, *636*, 282–298.

Berger, E., Dersch, G., Dellantonio, A., Duboc, O., Manner, K., Möbes-Hansen, B., & Stemmer, M. (2012). *Kupfer als Pflanzenschutzmittel - Strategie für einen nachhaltigen und umweltschonenden Einsatz (Copper as plant protectant – Strategy for sustainable and environmental-friendly use, in German)*. Austrian Agency for Health and Food Safety, Vienna.

Beye, F. (1974). Pesticides in the environment. *Biology in Our Time*, *4*(3), 80–86.

Bioland. (2022). Bioland eV; Bioland guidelines. Retrieved from: https://www.bioland.de/richtlinien

Bruus Pedersen, M., Axelsen, J. A., Strandberg, B., Jensen, J., & Attrill, M. J. (1999). The impact of a copper gradient on a microarthropod field community. *Ecotoxicology*, *8*(6), 467–483.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2022a). Verzeichnis zugelassener Pflanzenschutzmittel. Retrieved from: https://apps2.bvl.bund.de/psm/jsp/index.jsp

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2022b). Federal Office of Consumer Protection and Food Safety; Terminated authorisations of plant protection products. Retrieved from: https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/Beendete_PSM.html;jsessionid=7B5AED3FA463ABE65551B335E3DE6641.1_cid351?nn=11031326

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2022c). Federal Office of Consumer Protection and Food Safety; Sales quantities of active substances in plant protection products from 1987 to 2021. Retrieved from: https://www.bvl.bund.de/EN/Tasks/04_Plant_protection_products/01_ppp_tasks/02_ppp_AuthorisationReviewActSub/03_ppp_DomesticSalesExport/PPP_domesticSales_and_Export_node.html;jsessionid=FE36E22EF5448E3E395D0904B41E02E7.2_cid372?cms_thema=Domestic+sales+and+export

Bünemann, E. K., Schwenke, G. D., & Van Zwieten, L. (2006). Impact of agricultural inputs on soil organisms—A review. *Australian Journal of Soil Research*, *44*(4), 379–406.

Bundy, J. G., Sidhu, J. K., Rana, F., Spurgeon, D. J., Svendsen, C., Wren, J. F., Sturzenbaum, S. R., Morgan, A. J., & Kille, P. (2008). 'Systems toxicology' approach identifies coordinated metabolic responses to copper in a terrestrial non-model invertebrate, the earthworm *Lumbricus rubellus*. *BMC Biology*, *6*, 25.

Calderwood, L., Lewins, S., & Darby, H. (2015). Survey of northeastern hop arthropod pests and their natural enemies. *Journal of Integrated Pest Management*, *6*(1), 1–14.

Clark, J. F. (1902). On the toxic properties of some copper compounds with special reference to Bordeaux mixture. *Botanical Gazette*, *33*(1), 26–48.

Collins, D., Luxton, T., Kumar, N., Shah, S., Walker, V. K., & Shah, V. (2012). Assessing the impact of copper and zinc oxide nanoparticles on soil: A field study. *PLoS One*, *7*(8), e42663.

De Filippis, L. F., & Pallaghy, C. K. (1994). Heavy metals: Sources and biological effects. In L. C. Rai, J. P. Gaur, & C. J. Soeder (Eds.), *Advances in Limnology Series: Algae and Water Pollution* (pp. 31–77). E. Scheizerbartsche Press.

Deising, H. B. (2022). *Pilzlicher Sekundärmetabolismus, biologischer Pflanzenschutz und Lebensmittelsicherheit. Eine molekulargenetische Einschätzung (Fungal secondary metabolism, biocontrol and food safety: A molecular genetic assessment, in German)*. ALVA-Tagung.

Deising, H. B., Gase, I., & Kubo, Y. (2017). The unpredictable risk imposed by microbial secondary metabolites: How safe is biological control of plant diseases? *Journal of Plant Disease and Protection*, *124*(5), 413–419.

Demeter. (2022). Demeter e.V.; Guidelines 2022. Retrieved from: https://www.demeter.de/sites/default/files/richtlinien/richtlinien_gesamt.pdf

de Oliveira-Filho, E. C., Lopes, R. M., & Paumgartten, F. J. R. (2004). Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere*, *56*(4), 369–374.

Diesner, M., Groß, R., Helbich, M., Blepp, M., Bäuerle, P., & Bunke, D. (2014). *Kupfer im Bio-Landbau: Hintergrund, Herausforderungen und Handlungsempfehlungen (Copper in organic agriculture: Background, challenges and recommendations, in German)*. *Öko-Institut e.V.* Freiburg: Institut für angewandte Ökologie.

European Chemicals Agency. (2018). Peer review of the pesticide risk assessment of the active substance copper compounds copper(I), copper(II) variants namely copper hydroxide, copper oxychloride, tribasic copper sulfate, copper(I) oxide, Bordeaux mixture. *EFSA Journal*, *16*(1), e05152.

European Chemicals Agency. (2020). Approval of active substances. Retrieved from: https://echa.europa.eu/de/regulations/biocidalproductsregulation/approvalofactivesubstance

European Chemicals Agency. (2021). Registration dossier copper. Retrieved from: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15562/1/1

European Union Commission. (2018). Final renewal report for the active substances copper compounds finalised in the standing committee on plants, animals, food and feed at its meeting on 27 November 2018 in view of the renewal of the approval of the active substances copper compounds, as candidate for substitution, in accordance with regulation (EC) No 1107/2009. Retrieved from: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as_id=1221

Felgentreu, D., Herwig, N., & Hommel, B. (2017). Kupfergehalte in deutschen Obstbauregionen und deren Auswirkungen auf Regenwürmer (Copper contamination of German fruit production sites and their impact on earthworms, in German). *Öko-Obstbau*, *4*, 19–21.

Fernandes, J. C., & Henriques, F. S. (1991). Biochemical, physiological, and structural effects of excess copper in plants. *The Botanical Review*, *57*(3), 246–273.

Frampton, G. K., Jänsch, S., Scott‐Fordsmand, J. J., Rombke, J., & Van den Brink, P. J. (2006). Effects of pesticides on soil invertebrates in laboratory studies: A review and analysis using species sensitivity distributions. *Environmental Toxicology and Chemistry*, *25*(9), 2480–2489.

Gaw, S. K., Palmer, G., Kim, N. D., & Wilkins, A. L. (2003). Preliminary evidence that copper inhibits the degradation of DDT to DDE in pip and stonefruit orchard soils in the Auckland region, New Zealand. *Environmental Pollution*, *122*(1), 1–5.

Gisi, U. (2013). Mechanisms of action of fungicides. In H.‐M. Poehling & J.‐A. Verreet (Eds.), *Lehrbuch der Phytomedizin* (4th ed., pp. 436–445). Verlag Eugen Ulmer.

Gitzel, J., & Kühne, S. (2016). Status Quo der Forschung zu Minimierung und Ersatz kupferhaltiger Pflanzenschutzmittel in Deutschland (Current status of research into minimizing and replacement of copper‐containing pesticides in Germany, in German). *Journal für Kulturpflanzen*, *68*(5), 125–129.

Hernandez‐Jerez, A., Adriaanse, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Focks, A., Marinovich, M., Millet, M., Pelkonen, O., Tiktak, A., Topping, C., Widenfalk, A., Wilks, M., Wolterink, G., Conrad, A., & Pieper, S. (2021). Statement of the PPR panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP). *EFSA Journal*, *19*(3), 6498.

Itter, H., & Pabel, U. (2013). Toxicology of lead, copper and zinc. Symposium "Alles Wild?" Retrieved from: http://www.bfr.bund.de/cm/343/toxikologie-von-blei-kupfer-und-zink.pdf

Jänsch, S., & Römbke, J. (2009), *Einsatz von Kupfer als Pflanzenschutzmittel‐Wirkstoff: Ökologische Auswirkungen der Akkumulation von Kupfer im Boden (Use of copper as plant protectant: Ecological impact of copper accumulation in soil, in German)*. Federal Environment Agency.

Kaim, W., & Rall, J. (1996). Copper—A "modern" bioelement. *Angewandte Chemie*, *108*(1), 47–64.

Kamoun, S. (2003). Molecular genetics of pathogenic oomycetes. *Eukaryotic Cell*, *2*(2), 191–199.

Knauert, S., & Knauer, K. (2008). The role of reactive oxygen species in copper toxicity of two freshwater green algae. *Journal of Phycology*, *44*(2), 311–319.

Kühne, S., Roßberg, D., Röhrig, P., von Mering, F., Weihrauch, F., Kanthak, S., Kienzle, J., Patzwahl, W., & Reiners, E. (2016). Status Quo der Anwendung kupferhaltiger Pflanzenschutzmittel in der deutschen Landwirtschaft und dem Gartenbau. *Journal für Kulturpflanzen*, *68*(7), 189–196.

Kühne, S., Roßberg, D., Röhrig, P., von Mering, F., Weihrauch, F., Kanthak, S., Kienzle, J., Patzwahl, W., & Reiners, E. (2017). The use of copper pesticides in Germany and the search for minimization and replacement strategies. *Organic Farming*, *3*(1), 66–75.

Kühne, S., Strassemeyer, J., & Roßberg, D. (2009). Anwendung kupferhaltiger Pflanzenschutzmittel in Deutschland (Application of copper‐containing pesticides in Germany, in German). *Journal für Kulturpflanzen*, *61*(4), 126–130.

Kumar, V., Kalita, J., Misra, U. K., & Bora, H. K. (2015). A study of dose response and organ susceptibility of copper toxicity in a rat model. *Journal of Trace Elements in Medicine and Biology*, *29*, 269–274.

La Torre, A., Iovino, V., & Caradonia, F. (2018). Copper in plant protection: Current situation and prospects. *Phytopathologia Mediterranea*, *57*(2), 201–236.

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J.‐N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. *Agronomy for Sustainable Development*, *38*, 28.

Landwirtschaftliches Technologiezentrum Augustenberg. (2022). *Integrierter Pflanzenschutz im Erwerbsobstbau (Integrated disease control in fruit crops, in German)*. Retrieved from: https://ltz.landwirtschaft-bw.de/pb/,Lde/Home/Service/Broschueren+zum+Pflanzenschutz

Liang, Q., & Zhou, B. (2007). Copper and manganese induce yeast apoptosis via different pathways. *Molecular Biology of the Cell*, *18*(12), 4741–4749.

Liu, A., Cao, H., Yang, Y., Ma, X., & Liu, X. X. (2016). Combinational effects of sulfomethoxazole and copper on soil microbial community and function. *Environmental Science and Pollution Research*, *23*, 4235–4242.

Mackie, K. A., Muller, T., & Kandeler, E. (2012). Remediation of copper in vineyards: A mini review. *Environmental Pollution*, *167*, 16–26.

Madeo, F., Fröhlich, E., & Fröhlich, K. U. (1997). A yeast mutant showing diagnostic markers of early and late apoptosis. *The Journal of Cell Biology*, *139*(3), 729–734.

Merrington, G., Rogers, S. L., & Zwieten, L. V. (2002). The potential impact of long‐term copper fungicide usage on soil microbial biomass and microbial activity in an avocado orchard. *Australian Journal of Soil Research*, *40*, 749–759.

Mertens, J., Wakelin, S. A., Broos, K., McLaughlin, M. J., & Smolders, E. (2010). Extent of copper tolerance and consequences for functional stability of the ammonia‐oxidizing community in long‐term copper‐contaminated soils. *Environmental Toxicology and Chemistry*, *29*(1), 27–37.

Michalski, B. (2015). Risk assessment of copper for consumers based on monitoring data. *Reports from the Julius Kühn‐Institut*, *179*, 5–11.

Michaud, A. M., Bravin, M. N., Galleguillos, M., & Hinsinger, P. (2007). Copper uptake and phytotoxicity as assessed in situ for durum wheat (*Triticum turgidum durum* L.) cultivated in copper‐contaminated, former vineyard soils. *Plant and Soil*, *298*(1–2), 99–111.

Morel, N. M. L., Rueter, J. G., & Morel, F. M. M. (1978). Copper toxicity to *Skeletonema costatum* (Bacillariophyceae). *Journal of Phycology*, *14*(1), 43–48.

Oliveira‐Garcia, E., von Tiedemann, A., & Deising, H. B. (2021). The measure mix matters: Multiple‐component plant protection is indispensable for coping with the enormous genome plasticity and mutation rates in pathogenic microorganisms. *Journal of Plant Diseases and Protection*, *128*, 3–6.

Padrilah, S. N., Sabullah, M. K., Shukor, M. Y. A., Yasid, N. A., Shamaan, N. A., & Ahmad, S. A. (2018). Toxicity effects of fish histopathology on copper accumulation. *Pertanika Journal of Tropical Agricultural Science*, *41*(2), 519–540.

Panagos, P., Ballabio, C., Lugato, E., Jones, A., Borrelli, P., Scarpa, S., Orgiazzi, A., & Montanarella, L. (2018). Potential sources of anthropogenic copper inputs to European agricultural soils. *Sustainability*, *10*, 2380.

Paoletti, M. G., Sommaggio, D., Favretto, M. R., Petruzelli, G., Pezzarossa, B., & Barbafieri, M. (1998). Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Applied Soil Ecology*, *10*, 137–150.

Pascaly, M., Jolk, I., & Krebs, B. (1999). Kupfer—Die biochemische Bedeutung eines Metalls. *Chemistry in Our Time*, *33*(6), 334–341.

Riepert, F. (2009). Effects of copper contamination on selected indicators of the soil ecosystem. *Journal für Kulturpflanzen*, *61*(4), 131–139.

Rolke, Y., Liu, S., Quidde, T., Williamson, B., Schouten, A., Weltring, K.‐M., Siewers, V., Tenberge, K. B., Tudzynski, B., & Tudzynski, P. (2004). Functional analysis of H_2O_2‐generating systems in *Botrytis cinerea*: The major copper‐Zn‐superoxide dismutase (BCSOD1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. *Molecular Plant Pathology*, *5*(1), 17–27.

Sauve, S., Hendershot, W., & Allen, H. E. (2000). Solid‐solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden, and organic matter. *Environmental Science & Technology*, *34*(7), 1125–1131.

Schmitt, N. (1969). Over 100 years of chemical weed control: Contributions to the history of chemical weed control. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, *76*(5), 282–290.

Schwab, W. (2000). Pflanzenschutz: Gestern, Heute ‐ Morgen? *Pharmacy in Our Time*, *29*(2), 107–114.

Siafakas, A. R., Wright, L. C., Sorrell, T. C., & Djordjevic, J. T. (2006). Lipid rafts in *Cryptococcus neoformans* concentrate the virulence determinants phospholipase B1 and copper/Zn superoxide dismutase. *Eukaryotic Cell*, *5*(3), 488–498.

Singh, R. P., Kumar, S., Nada, R., & Prasad, R. (2006). Evaluation of copper toxicity in isolated human peripheral blood mononuclear cells and its attenuation by zinc: *Ex vivo*. *Molecular and Cellular Biochemistry*, *282*, 13–21.

Smolders, E., Oorts, K., van Sprang, P., Schoeters, I., Janssen, C. R., McGrath, S. P., & McLaughlin, M. J. (2009). Toxicity of trace metals in soil as affected by soil type and aging after contamination: Using calibrated bioavailability models to set ecological soil standards. *Environmental Toxicology and Chemistry*, *28*(8), 1633–1642.

Solioz, M. (2018). *Copper and bacteria. Evolution, Homeostasis and Toxicity. SpringerBriefs in Biometals* (1st ed.). Springer Nature.

Solomon, F. (2009). Impacts of copper on aquatic ecosystems and human health. Retrieved from: https://akmininginfo.files.wordpress.com/2012/09/solomon_impactscopperaquaticecosystemshumanhealth.pdf

Sunda, W., & Guillard, R. R. L. (1976). The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *Journal of Marine Research*, *34*(4), 511–529.

Tamm, L., Thuerig, B., Apostolov, S., Blogg, H., Borgo, E., Corneo, P. E., Fittje, S., de Palma, M., Donko, A., Experton, C., Alcázar Marín, É., Morell Pérez, Á., Pertot, I., Rasmussen, A., Steinshamn, H., Vetemaa, A., Willer, H., & Herforth-Rahmé, J. (2021). Copper use in organic agriculture in twelve European countries. *agriRxiv*, 2021. https://doi.org/10.31220/agriRxiv.2021.00108

von Tiedemann, A. (1997). Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. *Physiological and Molecular Plant Pathology*, *50*(3), 151–166.

United States Environmental Protection Agency. (2007). Aquatic life ambient freshwater quality criteria–Copper. EPA-822-R-07-001. https://www.epa.gov/sites/default/files/2019-02/documents/al-freshwater-copper-2007-revision.pdf

World Health Organization. (1996). *Trace Elements in Human Nutrition and Health*. Geneva.

Wilbois, K., Kauer, R., Fader, B., Kienzle, J., Haug, P., Fritzsche-Martin, A., Drescher, N., Reiners, E., & Röhrig, P. (2009). Kupfer als Pflanzenschutzmittel unter besonderer Berücksichtigung des Ökologischen Landbaus (Copper as plant protection product with special regards to organic farming, in German). *Journal für Kulturpflanzen*, *61*(4), 140–152.

Winkler, A., Athoo, T., & Knoche, M. (2022). Russeting of fruits: Etiology and management. *Horticulturae*, *8*(3), 231.

Yasokawa, D., Murata, S., Kitagawa, E., Iwahashi, Y., Nakagawa, R., Hashido, T., & Iwahashi, H. (2008). Mechanisms of copper toxicity in *Saccharomyces cerevisiae* determined by microarray analysis. *Environmental Toxicology*, *23*(5), 599–606.

Yu, S., Wang, Y., Shen, F., Fang, H., & Yu, Y. (2022). Copper-based fungicide copper hydroxide accelerates the evolution of antibiotic resistance via gene mutations in *Escherichia coli*. *Science of the Total Environment*, *815*, 152885.

Zhou, C.-F., Wang, Y.-J., Li, C.-C., Sun, R.-J., Yu, Y.-C., & Zhou, D.-M. (2013). Subacute toxicity of copper and glyphosate and their interaction to earthworm (*Eisenia fetida*). *Environmental Pollution*, *180*, 71–77.

Zhu, X., Gibbons, J., Garcia-Rivera, J., Casadevall, A., & Williamson, P. R. (2001). Laccase of *Cryptococcus neoformans* is a cell wall-associated virulence factor. *Infection and Immunity*, *69*(9), 5589–5596.