Polyoxin D Zinc Salt

Crops

Identification of Petitioned Substance

Chemical Names:

$C_{17}H_{23}N_5O_{14} \cdot Zn$

beta-D-allofuranuronic acid, 5-((2-amino-5-O-(aminocarbonyl)-2-deoxy-L-xylonoyl)amino)-1-(5-carboxy-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidinyl)-1,5-dideoxy-, zinc salt (1:1);

5-pyrimidinecarboxylic acid, 1-(5-(2-amino-2-deoxy-L-xylonamino)-5-deoxy-beta-D-allofuranuronosyl)-1,2,3,4-tetrahydro-2,4-dioxo-, monocarbamate (ester), zinc salt;

1-[(2R,3R,4S,5R)-5-[(S)-[[2S,3S,4S)-2-amino-5-carbamoyloxy-3,4-dihydroxypentanoyl]amino]-carboxymethyl]-3,4-dihydroxyoxolan-2-yl]-2,4-dioxopyrimidine-5-carboxylic acid;zinc

Other Names:

Polyoxorim-zinc
Polyoxorim Zinc Salt

Trade Names:

Veggieturbo 5SC Suspension Concentrate Fungicide
OSO 5%SC Fungicide
Endorse
Veranda O
Affirm WDG
Tavano 5%SC Fungicide
Novel 0.5%SC Fungicide

CAS Numbers:

146659-78-1
33401-46-6

Other Codes:

F48U67E18L (FDA UNII)
PC230000 (EPA Pesticide Chemical Code)
EPA Reg. No. 68173-1

Summary of Petitioned Use

Polyoxin D zinc salt currently appears on the National List of Allowed and Prohibited Substances (hereafter referred to as the "National List") as plant disease control at 7 CFR 205.601(i).

In January 2012, the National Organic Program (NOP) received a petition to add polyoxin D zinc salt to the National List for use in the control of fungal diseases including anthracnose, *Alternaria* diseases, downy mildew, powdery mildew, *Botrytis* diseases, and *Rhizoctonia* diseases in crops (Kaken Pharmaceutical Co. Ltd., 2012). The original petition also included post-harvest uses. The petitioner submitted an amendment to remove these additional uses in March 2012, because the Environmental Protection Agency (EPA) had not yet approved polyoxin D zinc salt as a post-harvest fungicide. The petitioner again submitted an amendment in October 2012 to include post-harvest uses after the EPA accepted their label.

In 2012, a technical report (USDA, 2012) was completed on the substance in support of the National Organic Standards Board (NOSB) review.

An additional petition amendment was submitted in January 2013, following the publication of the technical report. The petitioner took issue with the technical report because it neglected to discuss the previous petition amendments. The first amendment removed post-harvest references and also elaborated that the substance was admittedly synthetic, and the second described the EPA registration for the use of polyoxin D zinc salt on all food commodities. Since the technical report was published in September 2012 and the petition amendment to add post-harvest uses was in October, the report did not address post-harvest uses. Additionally, the petitioner disagreed with the technical report's characterization of polyoxin D zinc salt as an antibiotic. The petitioner considered many of the references cited in the report to be irrelevant because they were specific to other polyoxin compounds.

The NOSB voted not to add polyoxin D zinc salt to the National List in 2013 due to the availability of alternative substances and concerns regarding the fungicidal action on non-pathogenic soil fungi.

In 2016, the petitioner again submitted a petition to the NOP to add polyoxin D zinc salt to the National List, for similar uses (Kaken Pharmaceutical Co. Ltd., 2016). This second petition expanded upon the first and contained the results of studies conducted by the petitioner. These studies described the substance's impact on soil organisms and beneficial insects, as well as the efficacy on several crops. They also included efficacy comparisons with brand-name fungicides appearing on the Organic Materials Review Institute (OMRI) Products List©. The petition included detailed information regarding the mode of action of polyoxin D zinc salt, and the petitioner's view of its advantages over other synthetic and nonsynthetic fungicides permitted in organic production. The petitioner posited that the substance was essential for organic producers due to its low toxicity, the lack of alternative products for certain pathogens, and the unique mode of action leading to reduced fungicide resistance concerns.

A limited scope technical report was published December 12, 2017 (USDA, 2017a) to support the original technical report and the NOSB's review of the substance. The NOSB voted to add polyoxin D zinc salt to the National List as a plant disease control material on April 27, 2018 citing the lack of alternatives for certain pathogens (based upon public comments from organic producers), the lack of negative impact on soil organisms, and public commenters' statements of its essentiality (NOSB, 2018). The NOP formally added polyoxin D zinc salt to the National List on November 22, 2019 (NOP, 2019).

In conjunction with the previous technical reports, this limited scope report supports the 2022 sunset review of polyoxin D zinc salt. The NOSB Crops Subcommittee identified three focus questions for this limited scope report:

1. Is there currently or is there a potential for fungal or other microbial resistance to polyoxin D Zinc salt in addition to strains of *Alternaria alternata* mentioned in the first TR?
2. Is there any current evidence for, or the potential for, direct or cross resistance in human pathogens resulting from the use of polyoxin D zinc salt?
3. Is polyoxin D zinc salt classified as an antibiotic, and if so, on what basis?

Characterization of Petitioned Substance

Composition of the Substance:

The polyoxins are a group of molecules consisting of a chain of amino acids attached to a five-carbon sugar and a nitrogen-containing base (known as peptidyl nucleosides), that can be isolated from the fermentation broth of *Streptomyces* spp. (Isono, Asahi, & Suzuki, 1969). Polyoxin A through polyoxin L all share the same characteristic molecular structure but are differentiated by specific functional groups. Polyoxin D contains a carboxylic acid functional group attached to the uridine nucleoside moiety (see Figure 1).

Polyoxin D is extremely soluble in water. To limit polyoxin D from washing away, manufacturers formulate it with zinc in order to increase retention on plant surfaces when used as an agricultural fungicide (Rahman, et al., 2017).

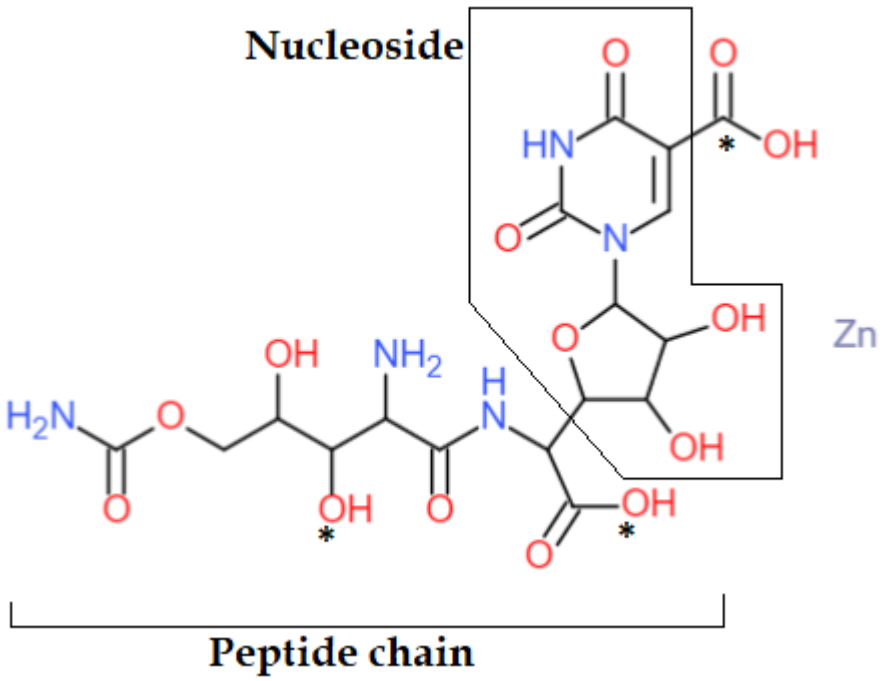

Figure 1: Structure of Polyoxin D zinc salt. The asterisks denote functional groups that differ depending on the polyoxin variety.

Source or Origin of the Substance:

Several varieties of naturally occurring polyoxin exist, designated alphabetically as polyoxin A through polyoxin L and differentiated by varying combinations of three functional groups attached to a shared molecular structure (Isono, Asahi, & Suzuki, 1969). This group of compounds can be isolated from the fermentation broth of *Streptomyces cacaoi* var. *asoensis* (Isono, Nagatsu, Kobinata, Sasaki, & Suzuki, 1967; Isono, Asahi, & Suzuki, 1969). As a crude extract, this mixture of polyoxins is typically referred to as "polyoxin AL". Each specific polyoxin molecule can be purified using a variety of chemical, chromatographic, and fractionation methods (Isono, Nagatsu, Kobinata, Sasaki, & Suzuki, 1967). An early isolation of polyoxin D involved ion exchange columns, elution (washing the target substance off) with sodium chloride, and chromatographic[1] separation through cellulose (Isono, Nagatsu, Kobinata, Sasaki, & Suzuki, 1967). Based on a review of available literature, the isolation and purification methods currently used to manufacture the EPA registered technical grade[2] polyoxin D, or polyoxin D technical, appear to remain confidential trade secrets of the original petitioning company, Kaken Pharmaceutical Co., Ltd.

Specific Uses of the Substance:

Agricultural Use

Polyoxins are used to control fungal diseases but are generally ineffective at controlling bacteria and yeasts (Copping & Menn, 2000). For example, polyoxin B is used to control *Alternaria* spp. such as pear black spot and apple cork spot, molds caused by *Botrytis cinerea*, rice sheath blight caused by *Rhizoctonia solani,* and for various fungal infections of turfgrasses (Copping & Menn, 2000). There do not appear to be any other

[1] Chromatography includes a variety of processes used to separate or purify substances by how fast they move through a medium (Lederer & Lederer, 1953).

[2] The EPA defines a technical grade active ingredient (or TGAI) as a "pesticide chemical in pure form (with impurities) as it is manufactured by a chemical company prior to being formulated into other pesticide products" (US EPA, 2021a)

significant commercial uses for polyoxin compounds at the present time, except as precursor chemicals in the development of new antifungal substances (Serpi, Ferrari, & Pertusati, 2016).

Researchers are not in agreement on which fungal diseases polyoxin D is effective against. Jones, Korir, Walter, & Everts (2020) found that polyoxin D zinc salt was moderately effective against gummy stem blight (*Stagonosporopsis* spp.) in cantaloupe and honeydew melon, and against anthracnose (*Colletotrichum orbiculare*) in watermelon. However, they found that it was not effective in controlling powdery mildew (*Podosphaera xanthii*) in these three crops during the course of their three-year field study. By contrast, Keinath (2016) found that polyoxin D zinc salt *was* effective against powdery mildew (as well as gummy stem blight) in melon seedlings, but *ineffective* against anthracnose in greenhouse settings.

The polyoxin D zinc salt labels registered with the EPA contain instructions pertaining to essentially every commercial crop, including berries, stone fruits, pome fruits, citrus, cucurbits, tubers, brassicas, bulb vegetables, greens, legumes, tree nuts, cereal grains, herbs, oilseeds, and fruiting vegetables (US EPA, 2017a; US EPA, 2020). The application instructions include uses as foliar spray, for in-furrow application, and for chemigation. Certain brand names also contain instructions for use on ornamentals and residential turf (US EPA, 2014a). The EPA has also accepted labels indicating post-harvest treatments for pome fruit, stone fruit, and pomegranate (US EPA, 2014b).

Human use

Considerable research has focused on polyoxins as less toxic alternatives to currently available therapeutic antifungal medications in humans (Serpi, Ferrari, & Pertusati, 2016). These studies have led to mostly unsuccessful results, and polyoxins are not used clinically at the present time. Polyoxin C is sometimes used as a precursor in the development of synthetic analogs used for efficacy research in pharmacological studies (Serpi, Ferrari, & Pertusati, 2016). See *Focus Questions* #*2* and #*3* for additional details regarding clinical uses for chitin synthase inhibitors.

Approved Legal Uses of the Substance:

Polyoxin D zinc salt technical is registered with the EPA for use on all food and feed crops (pre-harvest and post-harvest), ornamentals, golf courses, residential lawns, parks and commercial and institutional grounds (US EPA, 2017b). The technical grade active ingredient was originally approved by the EPA in 1997 for use only on golf course turf, residential lawns and commercial and institutional grounds (US EPA, 1997a). The EPA later approved its use on several food crops (EPA, 2008), and later for all food and feed crops pre- and post-harvest (US EPA, 2012a). The EPA has since approved numerous brand-name labels (see "identification of petitioned substance" above) that describe instructions for extensive crop and fungus pathosystems.

Under the EPA's *Exemptions From Tolerances,* 40 CFR §180.1285 (2012), polyoxin D zinc salt is exempt from the requirement of a tolerance for residues in or on all food commodities when applied as a fungicide and used in accordance with good agricultural practices.

Action of the Substance:

Polyoxin D zinc salt has a unique mode of action when compared to other commonly used conventional fungicides, and fungicides used in organic production. The polyoxins group acts on chitin synthase enzymes as described below. The Fungicide Resistance Action Committee groups fungicides by their biochemical mode of action to help identify resistance patterns across different active ingredients, assigning them a numbered designation that typically appears at the top of pesticide labels. This is intended to act as a simple reference identifier to inform operators to plan rotations of fungicides by differing numbers. Polyoxins are identified as FRAC Code 19 (Fungicide Resistance Action Committee , 2021). No other fungicide is defined as FRAC Code 19, indicating that cross-resistance with other fungicide types is unlikely.

Fungi produce cell walls containing chitin. Chitin is a polymer formed by joining monomers of the modified sugar *N*-acetylglucosamine into chains (Dutta, Dutta, & Tripathi, 2004). In order to produce this modified sugar, enzymes break apart a larger molecule, UDP-*N*-acetylglucosamine (Ohta, Kakiki, &

Misato, 1970; Raimi, et al., 2020). The structure of polyoxin D is similar (but not identical) to that of UDP-*N*-acetylglucosamine. Polyoxin D inhibits chitin synthase enzymes, which prevents the formation of chitin in the cell walls of fungi (Cabib, 1991; Zhang & Miller, 1999). Lacking sufficient chitin, fungal cells exposed to polyoxin D swell, burst and are unable to divide and multiply (Becker, Covert, Shenbagamurthi, Steinfeld, & Naider, 1983).

Because polyoxin D affects chitin formation, it is ineffective against bacteria and nontoxic to mammals and plants, because these organisms do not contain cell walls with chitin (Copping & Menn, 2000; Zhang & Miller, 1999). Unlike most mammals and other vertebrates, invertebrate animals (such as insects) do contain chitin. The effect of polyoxins on insects is discussed below (see *Evaluation Question #8*).

In order to be effective, polyoxins may need to be transported into the fungal cell. Several researchers have proposed that polyoxins enter the fungal cell membrane through a peptide transport system. The presence of other peptides (as would be found in live organisms) interferes and prevents transport into the cell, rendering polyoxin essentially ineffective (Emmer, Ryder, & Grassberger, 1985; Hector, 1993; Mehta, Kingsbury, Valenta, & Actor, 1984). While several attempts have been made to create synthetic polyoxin analogs to utilize these peptide transport systems, there appears to have been limited success so far (Chaudhary, Tupe, & Deshpande, 2013; Serpi, Ferrari, & Pertusati, 2016). As polyoxin D does not easily penetrate the cytoplasmic membrane of all cells, it has thus far been ineffective in therapeutic exploratory studies for potential human use, except at very high concentrations (Cabib, 1991; Emmer, Ryder, & Grassberger, 1985).

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

Evaluation Question #8: 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)).

Previous technical reports have touched upon the effect of polyoxins on non-target insect species. The 2012 report noted that polyoxin D inhibited a chitin-forming enzyme in cockroaches (USDA, 2012). The 2017 limited-scope report noted that results from studies on a variety of invertebrates were unreliable (noted by EPA as "disregarded"), with the exception of a study on earthworms (USDA, 2017a). Based on the report, results from earthworms indicated that the lethal concentration was above the application rate, such that the EPA deemed the effects to be below the level of concern.

Additional research into the use of polyoxins as potential insecticides indicates that they can be toxic, but only under certain circumstances that are unlikely to occur during field application (Arakawa, Yukihiro, & Noda, 2008; Vardanis, 1978). These studies focus on insect pest species. The data, however, should be applicable to many other insects.

The hydrophobic (water-repelling) nature of insect exoskeletons prevents the absorption of the polar (hydrophilic) polyoxin molecules, and they may be broken down into inactive forms within the digestive tract (Cohen, 2010). Though the literature is more limited regarding insecticidal aspects of polyoxin when compared to fungicidal uses, there appears to be little evidence that insect exposure by contact results in significant injury.

Using various larval stages of armyworms and cutworms, Arakawa, Yukihiro & Noda (2008) showed that polyoxin AL (but predominately polyoxin B by concentration) was effective against some Lepidoptera[3] species orally or by contact. However, isolated polyoxin D was efficacious only when directly injected into tissues. This indicated that application of isolated polyoxin D as an agricultural fungicide would not affect the life cycle of Lepidoptera. The authors suspect that polyoxin B may be the actively insecticidal analog

[3] Lepidoptera is the taxonomic order that includes butterflies and moths.

affecting Lepidoptera, though they also concede that their samples of polyoxin B had significant impurities of other polyoxin compounds and additives.

Vardanis (1978) reached a similar conclusion but used grasshoppers instead of Lepidoptera. When injected into the tissue of immature insects, polyoxins A and D were lethal. Individuals typically died during molting, as the new exoskeleton was not strong enough due to a lack of chitin. Topically applied, polyoxins A and D were largely not toxic to nymphs. However, it changed adult wing patterns when it was applied to nymph wing pads during development. Adults with fully formed exoskeletons were wholly unaffected by polyoxins, even when injected. Jyothi et al. (1985) also found similar effects on immature insects as Vardanis. When injected with polyoxins at the 5th instar[4] developmental stage, nymphs of *Dysdercus cingulatus* (a pest of cotton crops) died during molting.

In experiments on cockroach leg regeneration, exposure to polyoxin D in the culture medium was shown to inhibit chitin synthesis in regenerative tissue cultures grown in artificial nutrient media (Sowa & Marks, 1975).

Focus Questions Requested by NOSB Crops Subcommittee

Focus Question #1: Is there currently or is there a potential for fungal or other microbial resistance to Polyoxin D zinc salt in addition to strains of *Alternaria alternata* mentioned in the first TR?

Very few studies have been published specifically on resistance to polyoxin D zinc salt (Rudland, 2020). Several studies on fungicide resistance to other polyoxin varieties exist in the form of conference proceedings and University bulletins in Japan from the 1970s-1980s, but many remain unavailable except in Japanese. A rough translation from one study describes occurrences of polyoxin resistance (type unspecified) in *A. alternata* in several apple orchards in Japan (Kawasaki, Mametsuka, & Tashiro, 1990). Based on the literature, microbial resistance to polyoxin D zinc salt is possible, although the path to that resistance remains unclear.

Polyoxin D zinc salt is a locally systemic fungicide (a fungicide that is mobile in a plant but does not have significant effect outside of the area of absorption). According to Clarke, Vincelli, Koch, & Munshaw (2020), all systemic fungicides carry some risk of resistance. They rated polyoxin D zinc salt as medium risk for resistance, the same resistance risk rating as numerous other conventional, synthetic fungicides. Of the potentially nonsynthetic fungicides (or synthetic fungicides that may be permitted in organic agriculture), described in their ratings charts, *Bacillus licheniformis, B. subtilis,* hydrogen dioxide, *Pseudomonas chlororaphis Reynoutria sachalinensis* plant extract, *Trichoderma harzianum,* and mineral oil, all carry a low-risk rating and are not classified with a FRAC code.

Several studies show that *Alternaria alternata* can develop resistance to polyoxin B (Adachi, Watanabe, & Tsuge, 1996; Copping & Menn, 2000). Some of the literature (including the 2012 technical report) has proposed that cross-resistance to polyoxin D zinc salt is possible due to a similar mode of action as polyoxin B (Hao, Hu, Chen, & Schnabel, 2017; USDA, 2012). Though the modes of action and the chemical and physical properties are similar, polyoxins A through L all have different antifungal activity, possibly due to functional groups attached to the main molecular structure affecting transport into the cell (Debono & Gordee, 1994; Mamiev, Korolev, & Elad, 2013). In a greenhouse study using sweet basil, Mamiev et al. (2013) showed that despite the development of low-level resistance to polyoxin AL, *Botrytis cinerea* (a target species on several EPA registered labels of polyoxin D zinc salt products) never developed strong resistance after 10 years of applications. They concluded that polyoxins are a low-risk fungicide for resistance development.

[4] As insects grow, they must shed their exoskeletons several times. Each stage of exoskeleton molting is denoted as an "instar."

The absence of high-level resistance development may be due to reduced fitness of polyoxin resistant mutant strains. Following prolonged use of polyoxins in Japan and Korea on apple orchards, significantly high resistance to polyoxin B was observed among *Alternaria mali* populations in the field (Hwang & Yun, 1986). This strong resistance persisted when fungal isolates cultured in a laboratory were successively transferred in the absence of fungicide. The acquired resistance appeared to reduce the rate of asexual spore formation, sporulation, and growth of mycelia, indicating that the resistant strains may not be competitive with the remaining sensitive strains after fungicide application ends in the field. Using polyoxin D, this phenomenon was also described for early blight (*Alternaria solani*) on potatoes, and damping-off fungi (*Sclerotium rolfsii)* on mustard in India (Maria & Sullia, 1986).

Dowling et al. conducted a study on detached apple, strawberry, and tomato fruits that were inoculated with various *Botrytis cinerea* isolates collected from five U.S. states (2016). The researchers found that 6.3% of the *B. cinerea* strains isolated exhibited reduced sensitivity to polyoxin D zinc salt. The researchers concluded that low-level polyoxin D zinc salt resistance may exist within the gray mold population. Further research indicated that a newly described species, *Botrytis fragariae,* made up the majority of polyoxin D zinc salt resistant strains (Dowling, Hu, & Schnabel, 2017; Dowling, Hu, & Schnabel, 2018). It appeared to be largely limited to strawberry blossoms and was only identified in the eastern United States and Germany. Dowling et al. (2018) noted that the *Botrytis* spp. isolates used in these studies were acquired from fields that had never been treated with polyoxin D zinc salt or other fungicides, indicating the possibility of a pre-existing natural genetic resistance rather than an entirely new acquired resistance.

One *Botrytis mali* isolate from a Coachella Valley, California strawberry field in 2016 exhibited reduced sensitivity to polyoxin D zinc salt, as well as resistance to fludioxonil and several other conventional fungicides (Cosseboom, Ivors, & Schnabel, 2018). Fludioxinil is a FRAC Code 12 fungicide, which means that it has a different mode of action than polyoxin D zinc salt (Fungicide Resistance Action Committee , 2021). Cosseboom et al (2018) found that fludioxinil had been applied to the strawberry field, but polyoxin D zinc salt had not, again suggesting a possible pre-existing natural genetic resistance. This was the first reported instance of gray mold caused by *B. mali* in the state of California, the United States' largest strawberry producing state (Samtani, et al., 2019).

Botrytis spp. cause a range of pathological conditions including damping-off disease, blossom and fruit infections, leaf blight, and post-harvest rots (Agrios, 2005). Researchers have indicated that the taxonomic classification of *Botrytis* spp. remains underdeveloped (Garfinkel, Coats, Sherry, & Chastagner, 2019), which complicates the task of assigning risk for fungicide resistance. *Botrytis* species respond differently to fungicides in terms of the incidence of resistance (Dowling, 2018). Due to the coexistence of several genetically diverse *Botrytis* species, sometimes even within the same field or plant, it is difficult to apply resistance management rotation paradigms effectively (Hu, Dowling, & Schnabel, 2018).

Several studies have proposed that polyoxin resistance is a function of decreased cell membrane permeability, rather than an alteration of the chitin synthase enzyme attachment site (see *Action of the Substance*, above) (Debono & Gordee, 1994; Hori, Eguchi, Kakiki, & Misato, 1974; Keller & Cabib, 1971; Dekker, 1976). Since the chitin synthase site resides within the cytoplasmic membrane of the cell, it is proposed that polyoxin resistance in *Alternaria kikuchiana* and *Saccharomyces carlsbergensis* strains is not due to enzyme alteration, but rather due to a decreased concentration of the fungicide within the cell membrane where the chitin synthase enzyme resides (Debono & Gordee, 1994; Hori, Eguchi, Kakiki, & Misato, 1974; Keller & Cabib, 1971).

Dekker (1976) studied polyoxin resistance in pear black spot caused by *A. kikuchiana* after observing decreased efficacy following a short period of application. When isolating fungicide-sensitive and fungicide-tolerant strains in a cell-free system[5], the research indicated that there was no difference in chitin synthase inhibition, again supporting the idea that resistance to polyoxin is actually a function of cell membrane permeability in intact cells instead of alteration of the chitin synthase enzyme (Dekker, 1976).

[5] Cell-free systems are important research tools, in which internal components of cells can be manipulated without interference from the complex interrelated processes of the living system, including transport channels in the cell wall.

In some cases, resistant populations may become sensitive again after application of a systemic fungicide has ceased, as observed in *A. kikuchiana* when exposed to polyoxins (Dekker, 1976; Kohmoto, 1974). The rate at which resistance reappeared again after resuming fungicide use was not explored.

While the EPA permits the use of polyoxin D zinc salt to control *Rhizoctonia* crown and root rot (*Rhizoctonia solani)* in sugar beets (Kaken Pharmaceutical Co. Ltd., 2020), only one study regarding its efficacy in this particular crop/pathogen system was found. The researchers found that polyoxin D was effective in reducing the severity of disease (Bolton, Panella, Campbell, & Khan, 2010). Resistance risk was not explored in this study.

Focus Question #2: Is there any current evidence for, or the potential for, direct or cross resistance in human pathogens resulting from the use of polyoxin D zinc salt?

Very few studies could be located directly related to polyoxin resistance in human pathogens. Given that polyoxins are inactive against bacteria, no research was found describing any correlation between the use of polyoxins and the occurrence of bacterial pathogens in humans.

While there are as many as 6 million identified species of fungi, only 600 or so are known to be associated with human physiology, and the majority of those are not responsible for infectious diseases (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019). Most fungal infections result from a very limited number of genera, including *Aspergillus*, *Candida*, *Cryptococcus*, *Pneumocystis*, *Histoplasma*, *Coccidioides*, and *Blastomyces,* as well as species in the order Mucorales (known as mucormycetes) (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019). Animal physiology typically presents an inhospitable environment for fungi, preventing runaway infection. Immunocompromised individuals such as HIV/AIDS patients, those undergoing cancer therapy, and those whose fungus suppressing bacterial flora have been compromised by the use of antibiotics are at particular risk (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019).

Direct Resistance

Of the fungi known to be associated with human health, many are unaffected by polyoxin D. As spores or mycelia, none of the following fungi appear to be affected by any of the polyoxins (Isono, Nagatsu, Kobinata, Sasaki, & Suzuki, 1967; Makins, Holt, & Macdonald, 1980):

- *Aspergillus* - molds that can cause severe and often lethal illness in immunocompromised individuals (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019).
- *Candida* - yeasts that cause thrush and vaginal infections (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019). More rarely, they invade the bloodstream, causing serious life-threatening infections.
- Mucormycetes - cause of rare, but serious illnesses of the organs and skin (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019).

Acquired resistance would not be expected in fungi that are unaffected by polyoxin D.

Polyoxin D has been shown to affect some species related to human health, however. For example, on immature spherules of *Coccidioides immitis*, concentrations under 200 µg/ml of polyoxin D caused the cells to burst (Hector & Pappagianis, 1983). Over this concentration, cells died directly. At 50 µg/ml, polyoxin D reduced endosporulation, thus disrupting the fungal reproductive cycle (Hector, 1993). Hilenski, Naider & Becker (1986) observed similar results, noting disruption in cell wall structures in *Candida albicans,* leading to the failure of cells to properly separate. While cell walls still formed, the absence of chitin in the walls led to structural weakening, indicating that polyoxin D exhibits fungistatic[6] tendencies. Another study demonstrated that polyoxin D had fungistatic activity against *Cryptococcus neoformans* (Becker, Covert, Shenbagamurthi, Steinfeld, & Naider, 1983), an opportunistic pathogen that can cause meningitis in

[6] In a Draft Pesticide Registration Notice, the EPA defines a fungistat as "*A substance or mixture of substances that inhibits the growth of fungi in the inanimate environment. Because a fungistat is not potent enough to destroy fungi, its use is considered to be for aesthetic or cosmetic (non-public health) purposes only and not for public health related purposes*." (US EPA, 2012b).

immunocompromised patients (Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019). These studies did not test whether microorganisms were at risk of resistance to polyoxin D. As with bacteria and antibacterial substances, fungi can develop resistance to antifungal substances that they are exposed to (Serpi, Ferrari, & Pertusati, 2016).

Though exceedingly rare, invasive infections of *Saccharomyces cerevisiae* have been reported in the literature following treatments with probiotics, after antibiotic-induced diarrhea (Muñoz, et al., 2005). Bowers, Levin, & Cabib (1974) observed a decrease in abnormal cell development across several generations of *S. cerevisiae* treated with polyoxin D (indicating lessened effect of the fungistat) but could not conclude whether this was a function of acquired resistance or inactivation of the polyoxin compound.

Though unrelated to human health, one study indicated that the prevalence of rumen bacteria in sheep fed a diet mixed with polyoxin D actually increased, likely due to reduced competition in the gut (Cann, Kobayashi, Onoda, Wakita, & Hoshino, 1993). Rumen fungal populations were reduced, and protozoa increased, possibly as a result of the higher availability of nutrients. While some fungal resistance to polyoxin D was observed, the researchers suspected this was due to dipeptide compounds that compete with polyoxin D transport into cells (Emmer, Ryder, & Grassberger, 1985; Hector, 1993; Mehta, Kingsbury, Valenta, & Actor, 1984). *Focus Question #3* provides additional details regarding this concept.

Cross-resistance

The term cross-resistance refers to the ability of microorganisms to develop resistance to multiple treatments after developing resistance to one treatment, often as a result of similar antimicrobial modes of action. No scientific literature was found that directly tested whether polyoxins can create cross-resistance in fungi to other medically important drugs or vice versa. Chitin synthase drugs, like nikkomycins have a similar mode of action to polyoxin D, and therefore could be candidates to watch for cross-resistance.

Histoplasma, *blastomyces*, and *coccidioides* are capable of infecting otherwise healthy people by inhalation (Goughenour & Rappleye, 2017; Konopka, Casadevall, Taylor, Heitman, & Cowen, 2019). These genera can be particularly difficult to treat clinically (Goughenour & Rappleye, 2017). They exist in either thread-like hyphal forms or as yeasts, depending on the environment. Each form responds differently to treatment. Nikkomycin Z[7], an antibiotic with the same mode of action and general molecular structure as the polyoxins, has shown promise in treating these "dimorphic" (dual form) fungal infections of the lungs (Goughenour & Rappleye, 2017; Hector, Zimmer, & Pappagiannis, 1990; Nix, Swezey, Hector, & Galgiani, 2009). Again, research was not found that tested resistance in these pathogens to polyoxins or nikkomycin Z. Examples of studies evaluating cross-resistance to polyoxins caused by exposure to nikkomycin Z could also not be located. Nikkomycin Z has undergone safety trials in which adverse effects were not reported in humans (Nix, Swezey, Hector, & Galgiani, 2009). This drug could be medically important in the development of new antifungal medications.

Focus Question #3: Is Polyoxin D zinc salt classified as an antibiotic, and if so, on what basis?

A majority of the literature refers to polyoxin D zinc salt as a "peptidyl nucleoside antibiotic" (Isono, Asahi, & Suzuki, 1969; Cohen, 2010; Chaudhary, Tupe, & Deshpande, 2013).

The definition of "antibiotic" differs depending on the regulatory body defining it. Under the EPA's definition, it is an antibiotic. Under FDA and CDC definitions, polyoxin D is not an antibiotic.

EPA definition

The EPA defines an antibiotic as "*A metabolic product of one microorganism or a chemical that in low concentrations is detrimental to activities of specific other microorganisms. Examples include penicillin, tetracycline, and streptomycin. Not effective against viruses. A drug that kills microorganisms that cause mastitis or other*

[7] Nikkomycins are another variety of chitin synthase inhibitors that work on the same principle as the polyoxins, and have a similar molecular morphology (Cabib, 1991). Recently, there has been interest in synthesizing "hybrid" antibiotics from mixtures of polyoxin and nikkomycin components (called polyniks) in the interest of locating compounds with greater stability and antifungal activity, but research is ongoing (Li, Li, Tian, Niu, & Tan, 2011).

infectious disease" (US EPA, 2021b). The EPA's definition encompasses polyoxin D zinc salt as an antibiotic because it is a metabolic product of bacterial fermentation (*Streptomyces* spp.) and is detrimental to activities of specific other microorganisms (it inhibits fungal production of cell wall chitin). The original EPA approval of polyoxin D zinc salt technical for use in turf management refers to the substance as an antibiotic, a fungistat, and fungicide (US EPA, 1997b).

Centers for Disease Control and Prevention (CDC) definition

The CDC defines antibiotics as "*medicines that fight infections caused by bacteria in humans and animals by either killing the bacteria or making it difficult for the bacteria to grow and multiply*" (CDC, 2021). Polyoxin D zinc salt is not included in the CDC's definition because it is not a medicine and is ineffective against bacteria (Copping & Menn, 2000).

FDA definition

There does not appear to be a formal glossary definition of "antibiotic" in FDA regulations. Certain guidances allude to a definition however: "*Antimicrobial drugs include all drugs that work against a variety of microorganisms, such as bacteria, viruses, fungi, and parasites. An antibiotic drug is effective against bacteria. All antibiotics are antimicrobials, but not all antimicrobials are antibiotics*" (FDA, 2018). In this example, polyoxin D zinc salt is not an antibiotic when used as a drug because it does not work against bacteria. It is an antimicrobial. Research has been conducted on chitin synthase inhibiting antifungal compounds that could someday be used clinically, but polyoxin D zinc salt is not currently FDA approved as a drug.

Future potential as an antibiotic drug

Many antifungal medications exhibit toxicity to both fungal and mammalian cells, as they tend to work on cellular systems shared by fungi and animals (Hector, 1993; Joly, Bolard, & Yeni, 1992). Additionally, there has been a marked increase in recent years of patients contracting fungal infections due to immunodeficiencies caused by cancer treatment, AIDS, and autoimmune disorders (Chaudhary, Tupe, & Deshpande, 2013). Several fungal pathogens, such as *Candida* spp., *Aspergillus* spp., and *Cryptococcus neoformans* have begun to exhibit resistance to currently available antifungal drugs as well (Perea & Patterson, 2002). This has led to some urgency in developing new antifungal drugs, and preferably compounds less toxic to patients. Antifungals that work only against cellular processes of fungi are of great interest.

Antifungal drugs that specifically target chitin production (such as polyoxin D) are relatively unlikely to be toxic to human cells (Becker, Covert, Shenbagamurthi, Steinfeld, & Naider, 1983; Ramakrishnan, Rathore, & Raman, 2016). Researchers began studying polyoxin D and nikkomycins in the early 1980s for their potential as therapeutic agents with microbial suppression similar to that of beta-lactam antibiotics (such as penicillin), but acting on fungi instead (Hector, 1993). While lab trials resulted in inhibition of yeasts (including *Saccharomyces cerevisiae* and *Candida albicans*), researchers have found polyoxin D is largely ineffective when used in to control fungi in living animals (Chaudhary, Tupe, & Deshpande, 2013; Debono & Gordee, 1994; Emmer, Ryder, & Grassberger, 1985; Serpi, Ferrari, & Pertusati, 2016). This may be due to the mechanism by which polyoxins are transported inside fungal cells (see discussion of peptide transport, in *Action of the Substance*, above). In other words, it has not been a viable candidate for use as an antibiotic clinically thus far because it generally does not kill intact fungal cells.

Report Authorship

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