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Research Article

Evaluation of Ziram as an oral toxic bait chemical for control of grass carp *Ctenopharyngodon idella*

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Abstract

The grass carp, *Ctenopharyngodon idella,* is an invasive species in North America that has been recorded in 45 states with breeding populations in several major river basins. Established populations of grass carp have had cascading, negative effects on aquatic ecosystem structure and function. Oral piscicide baits have been examined as a potential method to manage invasive grass carp. Our goal was to examine the oral toxicity of the dimethyl-dithiocarbamate fungicide, Ziram, to grass carp. Three toxicity experiments used different carriers to deliver single Ziram doses ranging from 0.25 to 250 mg/kg by gavage. No acute mortality was observed when grass carp were gavaged with Ziram at the highest concentrations dissolved in ethanol at 40 mg/kg, suspended in dimethyl sulfoxide (DMSO) at 250 mg/kg, or suspended in polyethylene glycol (PEG) at 150 mg/kg. Ziram exposure through intraperitoneal injection resulted in acute mortality at 150 mg/kg potentially due to increased residence time in the peritoneal cavity and thereby greater opportunity for absorption. These results indicate that Ziram is acutely toxic to grass carp, however, additional research is required to formulate a successful novel grass carp toxicant that can be used to target the invasive species while minimizing effects on non-target fish species.

Key words: invasive carp, invasive species control, gavage, intraperitoneal injection, carrier

Introduction

The grass carp, *Ctenopharyngodon idella*, is one of the four invasive carp species established in North America (Conover et al. 2007). The other invasive carp are bighead carp (*Hypophthalmichthys nobilis*), black carp (*Mylopharyngodon piceus*), and silver carp (*Hypophthalmichthys molitrix*). Grass carp are originally from eastern Asia, with a native range from northern Vietnam to the Amur River on the Siberia-China border (Cudmore and Mandrak 2004). In the United States, grass carp have been recorded in 45 states with breeding populations in several major basins including the Missouri and Mississippi rivers (Burr and Warren Jr 1986; Chapman et al. 2013; Courtenay Jr. 1993). Grass carp are primarily herbivorous; up to 95% of their adult diet is composed of aquatic vegetation (Fedorenko and Fraser 1978). They were introduced to North America as a low-cost,

non-chemical approach for aquatic plant control in an attempt to improve fisheries habitat (Courtenay Jr. et al. 1984; Cudmore and Mandrak 2004; Pierce 1983; Trent et al. 1992). However, established populations of grass carp have had cascading negative effects on aquatic ecosystem structure and function. For example, ecosystems invaded by grass carp have decreased aquatic plant numbers and density, a reduced ability to absorb nutrients, loss of native fish species habitat, reduced food for waterfowl, and increased incidence of algal blooms (Bain et al. 1990; Lembi et al. 1978; Leslie Jr. et al. 1987; McKnight and Hepp 1995; Pipalova 2006; Trent et al. 1992; van Dyke et al. 1984).

Efforts to manage invasive fish have examined a variety of techniques including chemical control, physical removal, biological control through introduction of predatory species, species-specific diseases, and recombinant DNA to produce sterile fish (Kolar et al. 2010). Chemical control of invasive fish using the piscicides antimycin-A and rotenone, commonly used as an aqueous treatment, has been investigated for use through the incorporation and application of the chemical in a bait or feed specific to common carp *Cyprinus carpio* and grass carp (Fajt and Grizzle 1993; Poole et al. 2018; Rach et al. 2009). Once ingested the piscicide is absorbed through the gastrointestinal (GI) tract. This direct ingestion can selectively target the invasive fish with minimal effects on non-target species. Studies using antimycin-A in the laboratory and mesocosm ponds found selective sensitivity in common carp when incorporated into a corn-based bait (Poole et al. 2018). Rotenone incorporated into an alfalfa pellet was successful in removing approximately 17% of stocked triploid grass carp in a field trial in Florida (Mallison et al. 1995). To encourage selective feeding on toxic bait, the fish were trained using a non-treated fish food and then switched to the rotenone bait. Results of field applications using the rotenone bait were (or have been) mixed with reports of minimal or no mortality (Bonneau and Scarnecchia 2001; Gehrke 2003; Mangan 2003). Poor performance of the bait was attributed to low rotenone palatability; feed training baits that lacked rotenone were more readily consumed than those that included rotenone. Therefore, there is a need to identify other toxicants that can be used for selective control of invasive grass carp.

Candidate piscicide-bait formulations should show some species-specificity, be palatable to target species, demonstrate low persistence in aquatic systems after application, and have minimal adverse effects on non-target species. One such chemical is Ziram (Supplementary material Table S1), a broad-spectrum pesticide belonging to the dimethyl-dithiocarbamate class of agricultural fungicides (Houeto et al. 1995; Kanchi et al. 2014). Dithiocarbamates are a class of complexing chemicals used in a wide range of industries including materials fabrication, mining, medicine, and agriculture (Nieuwenhuizen et al. 1999; Sauna et al. 2005). Dimethyl-dithiocarbamate is an organosulfur anion that is most frequently

complexed with cationic metals such as sodium, zinc, manganese, and copper. The biochemical effects of the dimethyl-dithiocarbamates are elicited through sulfhydryl binding of amino acids, proteins, and enzymes and chelation of essential metals required for enzymatic activity (Maitre et al. 1993). Binding of dimethyl-dithiocarbamates to proteins leads to alteration in function such as inhibition of superoxide dismutase leading to oxidative stress, inhibition of acetylcholine esterase, and stimulation of non-selective cation channels (Rath et al. 2011). Due to its potential toxicity to fish and potential selectivity for carp species, Ziram was selected for investigation as a potential piscicide bait toxicant.

The objective of this study was to quantify the level of Ziram that will cause mortality (i.e., LD_{50} value) for use as a piscicide bait to selectively support the control of grass carp (Bonneau and Scarnecchia 2001; Mangan 2003). Initially, literature was reviewed to determine the potential selective toxicity of Ziram to grass carp compared to other fish species. This information was also used to determine the potential for unintended acute toxicity in non-target species. To investigate the oral toxicity of Ziram, three experiments of single-dose oral gavage and intraperitoneal injection toxicity experiments were conducted: 1) evaluating a single dose of Ziram, 2) evaluating the influence of the solvent carrier, and 3) evaluating the route of exposure of the Ziram. Test fish were dosed by oral gavage or intraperitoneal injection and observed following exposure to determine acute mortality, and time to mortality and to assess latent effects. This information is intended to guide future studies on the potential use of Ziram as a single-dose toxicant to specifically target and manage invasive grass carp populations.

Materials and methods

Development of species sensitivity distribution

The selective toxicity of Ziram to fish was evaluated by reviewing existing aqueous toxicity data in the literature. A ranked percentile distribution and species sensitivity distribution (SSD) was developed to determine the sensitivity of carp species relative to other non-target fish species. A literature search was conducted using Web of Science, Scopus, and Google Scholar. The resulting published papers were reviewed and screened using criteria to focus the ranked percentile distribution and include the most robust and relevant data (Steevens et al. 2005). In addition to the published papers, we also searched knowledgebases for studies reporting effect concentrations in fish. These databases included the U.S. Environmental Protection Agency (EPA) Ecotox Knowledgebase, U.S. EPA Office of Pollution Prevention Pesticide Ecotoxicity Database, California Department of Fish and Game Database, European Chemicals Agency, and Canada Pesticide Product Information Database. Data from the knowledgebases were only included

if it was deemed acceptable for use or meeting quality control requirements such that it was included in the regulatory assessment. All studies reported aqueous exposure to Ziram and often included the use of solvent carriers to enhance solubility or exposure (Table S1). Only studies that used Ziram were included in the data set. Data reporting 50 percent lethal aquatic concentration (LC50) values were included. Because of the limited number of studies that we found, those reporting either nominal concentrations and analytically confirmed stocks or chemical concentrations were included. Duration of exposure reported in the literature ranged from acute (i.e., 96 h or less) to chronic exposures greater than a week. However, due to the interest in using Ziram as a chemical in a piscicide bait we focused the ranked percentile distribution on acute exposures less than 96 h. A species sensitivity distribution of the data set in Table S2 was developed using the Causal Analysis/Diagnosis Decision Information System (CADDIS) SSD Generator V1 (U.S. Environmental Protection Agency 2016).

Chemical preparation

High purity analytical standard Ziram (zinc dimethyldithiocarbamate, 100 analytical standard grade, CAS number 137-30-4) was purchased from Sigma-Aldrich (St. Louis, Missouri). Ziram has a relatively low water solubility (0.97 to 65 mg/L; see Table S1). Therefore, Ziram stock solutions, prepared the day before each bioassay and stored at room temperature, were created by adding 800 mg of Ziram directly into the solvent carriers, ethanol and dimethyl sulfxide (DMSO), from Sigma, St. Louis, Missouri, and polyethylene glycol obtained from Bayer Global (Whippany, New Jersey). Polyethlene glycol (PEG) was formulated at 10% (wt/vol) in deionized water. Stock solutions used for gavage were analyzed by Pacific Agricultural Laboratory (Sherwood, Oregon) using U.S. EPA method 630 (U.S. Environmental Protection Agency 1993). Briefly, the method employs an acid digestion to generate carbon disulfide (CS_2), which is trapped in the headspace of the chamber, reacted with a color reagent (cupric acetate and diethylanolamine), and measured spectrophotometrically. This method was not successful in quantifying Ziram in the stock solutions due to interferences from the carrier/suspension solvent DMSO and PEG. While these interferences are noted in the standard method, we did not have adequate sample remaining for a solvent extraction and liquid chromatography–mass spectrometry analysis. Therefore, all doses reported nominal Ziram concentrations.

Test organisms

Diploid grass carp for the experiments were collected from rearing ponds at the U.S. Geological Survey Columbia Environmental Research Center (CERC). Fish were reared to a mean weight 62.4 ± 24.8 g and length of 175 ± 23 mm for use in all three studies.

Oral toxicity of Ziram to grass carp

Three experiments were conducted to determine the oral toxicity of Ziram in grass carp. The first two studies were range-finding studies to estimate the median lethal dose (LD50) of Ziram prior to conducting a definitive experiment to derive an LD50 used to guide bait development. The second study used DMSO as a carrier for Ziram and also included an assessment of potential leaching of Ziram into water after gavage. For this study bluegill (*Lepomis macrochirus*) were added to the tanks to monitor for potential non-target effects due to a soluble fraction of Ziram passing from the grass Carp. The third experiment was conducted to compare the toxicity of Ziram through oral gavage and intraperitoneal (IP) injection.

Experiment 1

The first experiment was conducted to estimate the dose of Ziram that causes acute mortality in grass carp. Toxicity screening methods using a reduced number of animals are recommended where little toxicity data are available in the literature (Walum 1998). Methods such as the limit test, fixed-dose procedure, toxic class procedure and up-and-down method can be used to determine no-effect levels (Lipnick et al. 995). In this study we chose to use a procedure where one animal replicate is used for multiple exposure concentrations to establish an effect level (Chinedu et al. 2013). Ziram doses were based on the high concentration of 40 mg Ziram/kg wet weight fish and a 50% dilution series of 20, 10, 5, 2.5, 1, 0.5, 0.25 mg Ziram/g, with a single fish dosed at each concentration. This minimized the number of fish required (10 total fish) and provided an estimate of the dose where acute effects occur, thereby eliminating the use of a large number of test organisms typically used in a standard bioassay (70 fish). Ziram was prepared in ethanol (5 mg/ml stock) and used for oral gavage.

The highest concentration used in this experiment was selected because it is the highest concentration of Ziram that was stable in ethanol. Stability was determined by preparing stock solution in the solvent carrier and mixing with sonication for a minimum of 10 minutes and observing the solution for up to 8 h for formation of colloids, particles, or any lack of clarity in the solution. Fish were transferred into 2,000 L flow-through and fasted for 24 h prior to use in the study. Fish used in the first bioassay were lightly anesthetized in approximately 60 mg/L tricaine methane-sulfonate (MS-222) until loss of equilibrium prior to gavage (Kroboth et al. *unpublished data*). Fish length and weight were recorded (Table S3), and fish were gavaged using an 18-gauge 76 mm reusable stainless-steel ball-tipped gavage needle (Pet Surgical, Phoenix, Arizona) attached to a 1 mL BD syringe (Becton, Dickerson and Company, Franklin Lakes, New Jersey). The needle was inserted into the GI tract beyond the pharyngeal teeth to administer each individual dose to a fish. Two negative control fish were

gavaged at the highest treatment volume of the ethanol carrier per gram of fish. Doses based on fish weight ranged from 0.14 to 8.0 µl ethanol/g. After the fish were gavaged, they were immediately placed into one of ten flow-through 40 L aquaria for observation. Water in the aquaria was renewed at a rate of 3 L/hr resulting in approximately two complete renewals per day. Mortality was monitored hourly for the first 5 hours of the study and then at 24 h. Mortality of grass carp was defined as a lack of operculum and general movement exceeding 5 minutes. Surviving test animals were euthanized with MS-222 after 24 h.

Experiment 2

Due to the limited stability of Ziram in ethanol, a second experiment was conducted using DMSO as a carrier. For this experiment, an 80 mg/ml stock suspension of Ziram was prepared in DMSO. In contrast to the Ziram prepared in ethanol, this stock was opaque suggesting the Ziram was not in solution but rather a suspension of colloids. Treatment doses were control, 50, 100, 125, 150, 175, 200, 225 and 250 mg Ziram/kg with a single fish dosed at each concentration. As in the first bioassay, fish were lightly anesthetized, measured for length and weight, and then gavaged with Ziram. The volume of DMSO used for dosing Ziram was based on a fish weight range from 0.61 to 3.1 µl/g. Two negative control fish were gavaged at the highest treatment volume of the DMSO carrier 3.1 µl/g. To determine if the carp were regurgitating or eliminating the chemical in feces, potentially resulting in an aqueous concentration lethal to non-target fish, we stocked two juvenile bluegill (mean length 5.3 ± 0.6 cm and mean weight 2.1 ± 0.9 g) in each test aquaria at 24 h. Bluegill were obtained from a culture maintained in the CERC research ponds. These fish were transferred into a 430 L holding tank until use. Bluegill were selected because of their sensitivity to Ziram (Douglas et al. 1991). Bluegill were not gavaged with Ziram, so any adverse effects would reasonably be assumed to be due to uptake through aqueous exposure to the Ziram expelled from the gavaged carp. Observations for mortality in carp were made for the first 5 hours of the study and then at 24 and 48 h while bluegill were checked at 48 h. After 48 h, surviving test animals were euthanized with MS-222.

Experiment 3

The third experiment was to determine to what extent the toxicity of Ziram was dependent on route of administration. In the first two experiments it appeared that, following oral exposure, Ziram was rapidly passed by the grass carp with little or no acute toxicity. Mobility of ingested materials in grass carp is rapid through the intestine and allows for a brief amount of time for uptake through the lumen. To determine the importance of uptake route of Ziram, a more direct route of delivery was used to

introduce the chemical internally where uptake is not dependent on absorption through the GI tract (Al Shoyaib et al. 2020). While IP injection is not a feasible route of exposure or delivery mechanism that will be used for grass carp applications, it does provide the internal dose of Ziram required to elicit acute mortality. This knowledge can be used as a design criterion for future dietary formulations in order to achieve an effective internal dose of Ziram. Therefore, we IP injected and gavaged grass carp with Ziram using DMSO and PEG as carriers at a dose of 150 ug Ziram/g. This concentration was selected because this was the level that Ziram appeared to be a stable suspension in PEG and DMSO above or near the upper limit of the previous two experiments. As in the first two bioassays, fish were lightly anesthetized prior to gavage or IP injection. Individuals were measured and weighed to determine the treatment dose based on the mass of the fish (1.3 to 4.5 ul/g fish wt, same for both routes). The amount of DMSO used in these studies (up to 4.5 ml/kg) is lower than the levels (6.45 ml/kg/day) that caused toxicity in salmon over a period of daily dosing for 28 days (Benville et al. 1968). Groups of five fish were gavaged or IP injected at a dose of 150 mg/kg using a stainless-steel needle to inject Ziram into the peritoneum. Control fish were dosed at the highest volume of DMSO or PEG carrier. Gavage exposure was implemented as described in experiment 1 and 2. Immediately after exposure the fish were placed in a 40-L aquarium for observation, and water in the aquaria was renewed at a rate of 3 L/h resulting in approximately two complete renewals per day. Fish were observed hourly for the first 6 h and then at 24 and 48 h. Data in the third experiment were evaluated using a survival curve analysis over time and plotted using a Kaplan-Meier survival curve (Machin et al. 2006). The Mantel-Cox test was used to compare survival curves and time to acute mortality between the treatment groups and solvent control (Bernabò et al. 2016; Machin et al. 2006; Roggenbeck et al. 2021; Vergauwen et al. 2013).

Results

Data for 13 species from 17 studies were used to generate the ranked percentile distribution and SSD for Ziram (Figure 1, Table 1). Numerous databases were queried to obtain adequate data points for the analysis. Only 2 of the 17 studies reported LC50 values for Ziram using measured exposure concentrations. All data used in the analysis were considered acute – 48 or 96 h exposure duration. Where more than one LC50 value was reported for a single species, the geometric mean was calculated and used in the distribution. These studies showed a wide range of sensitivity of fish ranging nearly 3 orders of magnitude. The lowest effect value reported in the literature and databases was 0.008 mg/L in a 96 h toxicity test with fathead minnow (*Pimephales promelas)* (Maloney and

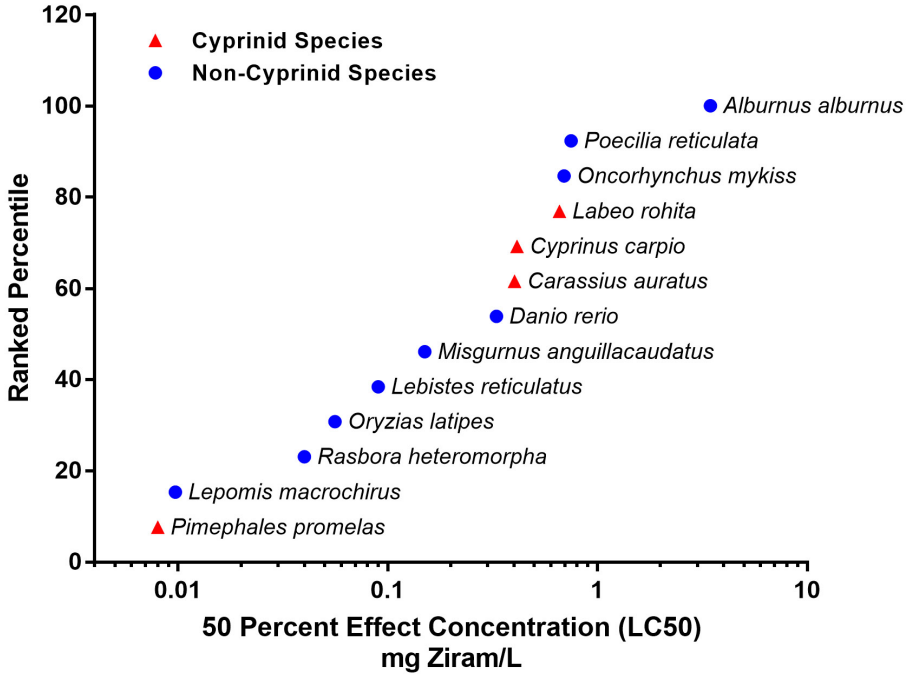

Figure 1. Ranked percentile for acute (≤ 96 h) median lethal concentration (LC50) values reported for fish exposed to Ziram in water. Data for the figure are provided in Table 1.

Palmer 1956). The second lowest 96 h LC50 value reported for fish was 0.0097 mg/L in bluegill (*Lepomis macrochirus*; Douglas et al. 1991; European Chemical Agency (ECHA) 2020). The bluegill study is considered a high-quality value because exposure concentrations were measured, a high purity standard was used, and it followed standard test guidelines. The highest LC50 value reported in any study was for the common bleak (*Alburmnus alburnus*) with a 96 h LC50 ranged from 3 to 4 mg/L (State of California 1999). Goldfish (*Carassius auratus*) had the second highest 96 h LC50 at 2.3 and 3.0 mg/L (State of California 1999; Van Wezel and van Vlaardingen et al. 2004). However, another study using goldfish reported an LC50 of 0.095 mg/L (Nishiuchi 1974). Therefore, the geometric mean for the two reported LC50 values used in the ranked percentile distribution and SSD was 0.47 mg/L. A geometric mean was also calculated for rainbow trout (*Oncorhynchus mykiss*) of 0.73, and the difference between values for those two studies was much smaller (Van Wezel and van Vlaardingen 2004; European Chemical Agency (ECHA) 2020). The acute LC50 for common carp (*Cyprinus carpio*) in a 48 h toxicity bioassay was 0.075 mg/L, making it the fourth most sensitive species (Nishiuchi 1974).

In our studies grass carp demonstrated limited toxicity to oral doses of Ziram. Control survival was 100% in the first two bioassays. Survival of grass carp gavaged with Ziram in the first two bioassays was 100% at all 8 concentrations tested in each bioassay (Table S3) at levels up to 250 µl/g in DMSO suspension. During each of these experiments it was noted that white feces, similar in color of the gavaged Ziram, on the bottom of test aquariums was present after 24 h. This indicates that Ziram gavaged into

Table 1. Acute (48–96 h) median lethal concentration (LC50) values reported for fish exposed to Ziram in water.

Species	Common Name	Study Duration (h)	Reported LC50 Value (mg/L)	Geomean (mg/L)	Chemical Analysis	Reference
Alburnus alburnus[1]	Common Bleak	96	3-4	3.46	NR	Linden et al 1979
Carassius auratus	Goldfish	96	2.3	0.40	NR	Van Wezel and van Vlaardingen et al. 1993
Carassius auratus	Goldfish	48	0.095	–	NR	van Wezel et al 2004; Reported by Nishiuchi 1974
Carassius auratus	Goldfish	96	0.3	–	NR	State of California, 1999; Conducted by Montedison USA
Cyprinus carpio	Common Carp	48	0.075	0.41	NR	Nishiuchi 1974
Cyprinus carpio	Common Carp	96	2.28	–	NR	U.S. EPA OPP Database; Conducted by Pharmatox Forschung & Betratung, Germany
Danio rerio	Zebrafish	96	0.33	–	NR	Cao et al. 2019
Labeo rohita	Rohu	96	0.66	–	NR	Sindhe et al. 2011
Lebistes reticulatus	Guppy	96	0.09	–	NR	State of California, 1999; Conducted by Montedison USA
Lepomis macrochirus	Bluegill	96	0.0097	–	Reported	European Chemical Agency, 2020; Conducted by Huntingdon Research Center, England
Misgurnus anguillacaudatus	Pond Loach	48	0.15	–	NR	van Wezel et al 2004; Reported by Nishiuchi 1974
Oncorhynchus mykiss	Rainbow Trout	96	0.27	0.69	NR	U.S. EPA OPP Database; Conducted by Pharmatox Forschung & Betratung, Germany
Oncorhynchus mykiss	Rainbow Trout	96	1.78	–	Reported	European Chemical Agency, 2020; Conducted by Huntingdon Research Center, England
Oryzias latipes	Japanese Medaka	48	0.056	–	NR	van Wezel et al 2004; Reported by Nishiuchi 1974
Pimephales promelas	Fathead Minnow	96	0.008	–	NR	U.S. EPA OPP Database; Maloney and Palmer, 1956
Poecilia reticulata	Guppy	96	0.75	–	NR	State of California, 1999; Conducted by Montedison USA
Rasbora heteromorpha	Harlequin Fish	96	0.04	–	NR	State of California, 1999; Conducted by Montedison USA

[1] LC50 value was reported as a range, therefore a geometric mean was calculated; Chemical analysis listed as reported or not reported (NR). Reference includes the source of information as well as identification of study reported in the assessment or database.

the stomach of grass carp passed through the GI tract and was likely evacuated with limited uptake. In the second experiment we evaluated the possibility that Ziram, evacuated from grass carp could partition into the aqueous phase and be toxic to bluegill. Bluegill survival in the second bioassay was also 100% in all test concentrations. Based on previous studies, the acute toxicity in bluegill occurs at approximately 0.0097 mg/L, and therefore we conclude that water soluble Ziram either regurgitated from the grass carp or through leeching from their feces in the test aquaria was minimal, less than 0.0097 mg/L or less than 0.03% based on mass balance for the highest concentration of Ziram.

In the third experiment we dosed grass carp by gavage and IP injection to determine what role the route of exposure might play in the toxicity of Ziram. Survival of grass carp in the control treatments was 100% for both carriers with gavage exposure, and 80% (1 death in 5 injected) and 100% when injected IP injection with the highest volume of the DMSO and PEG

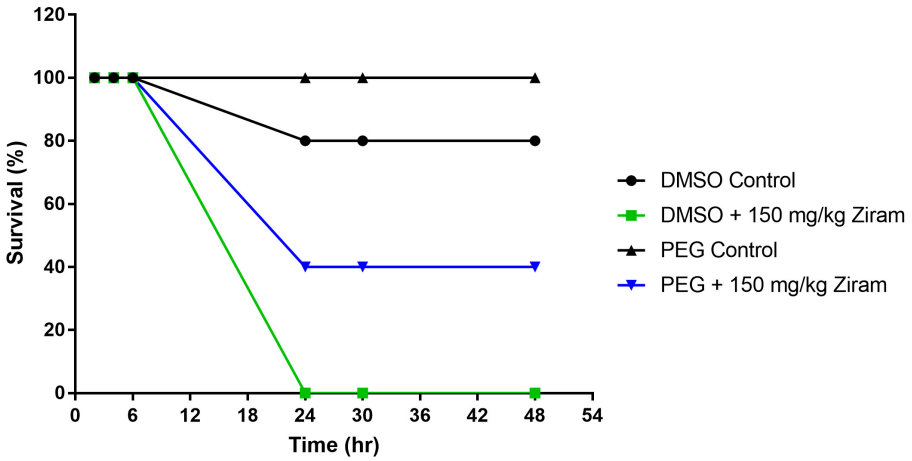

Figure 2. Kaplan-Meier time to mortality survival curve for sub-adult grass carp intraperitoneal injected with Ziram using dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG) carrier. Each treatment included n = 5 fish injected with carrier control or carrier + Ziram and then observed for 48 hours. Survival results over time were evaluated using the Mantel-Cox test. Survival curves for Ziram-treated fish were significantly different from their DMSO (P = 0.003) and PEG (P = 0.007) carrier controls.

carrier controls, respectively. The single mortality in the DMSO control group may be associated with the smaller fish (replicate 3, 22.9 g) that received a proportionally higher dose of DMSO carrier as compared to the other fish. The level of DMSO this fish received (10.9 µl/g) is above or near levels previously reported to cause mortality in trout and salmon over a 28-day period (Benville et al. 1968). However, the acute mortality observed here may also be due to injury that may have occurred at the site of the injection. A significant reduction in Ziram-dosed grass carp survival compared to the control was observed when the compound was administered via IP injection with either carrier. Survival following IP injection treatments was 0% and 40% with the DMSO and PEG carriers, respectively (Figure 2).

Discussion

The lack of toxicity to orally dosed Ziram may be due to gut evacuation rate in the grass carp of this study. We found that no acute mortality was observed in grass carp gavaged with Ziram dissolved in ethanol at 40 mg/kg or in DMSO at 250 mg/kg. In the third experiment we found that 150 mg Ziram/kg caused significant acute mortality in grass carp within 24 hours when administered via IP injection. The difference between gavage and IP injection suggests that internalization of Ziram is likely limited in the intestine, and therefore has limited acute toxicity. While the toxicokinetic parameters of Ziram in fish are not well known, it is plausible that the low toxicity of oral-dosed Ziram may be due to low GI tract uptake and rapid evacuation of the chemical. In the water-only exposures, the uptake of aqueous Ziram likely occurs across the gills or GI tract; because fish fasted for 24 h prior to gavage, it is likely the Ziram passed through the GI tract within 24 hours. The GI tract of grass carp, as in other herbivorous fish,

lacks a stomach and is relatively short compared to the GI tract of carnivorous fish. As a result, the residence time of food (and presumably toxicants) in grass carp, is approximately 8 to 18 h, depending on feeding rate and temperature (Stevens and Hume 1998). This brief residence time and conditions in the GI may limit uptake of Ziram and therefore, it can be inferred that Ziram effectively passes through the gut with little transport across the GI epithelium.

The species sensitivity distribution (Table S2) shows the relative sensitivity of carp species (*C. auratus, C. carpio, L. rhita*) to other fish species. The LC50 values for carp species range from 0.4 and 0.66 mg/L and are within the 58 to 73 percentiles of the distribution. It is expected the toxicity of Ziram to grass carp would be similar to other carp species in the data set. Therefore, an effective method to control invasive carp species through aqueous exposure would require a higher exposure level that is likely to affect non-target fish species. For example, a concentration likely to produce 100 percent acute mortality in grass carp would range from 2 to 10 times greater than the carp species LC50 value. A level of Ziram 10 times greater than geometric mean of LC50 value of three carp species (0.48 mg/L) is 4.8 mg/L Ziram. This level of treatment would be higher than the 95th percentile of LC50 values for all species identified in the SSD (3.68 mg/L; 95% confidence interval 1.54–8.77). In order to selectively control grass carp, a method is needed to minimize the effects on non-target species through targeting application such as through a dietary exposure.

A steady state bioconcentration study in Rainbow Trout found Ziram accumulated in liver and gut with very little in the gill or other tissues (Van Leeuwen et al. 1986). Absorption in rats was relatively slow with the maximum internalized level of Ziram observed after a single dose at 10 hours (Greim 2009). However, elimination by rats is relatively fast with a half-life of around 33 to 38 h. As a result, the peak body burden of Ziram in mammals following an oral dose is relatively small, and most of the chemical is rapidly eliminated. It is reasonable that following uptake in grass carp the kinetics would be similar and therefore Ziram would be rapidly eliminated. Other differences in uptake may be a result of the low pH in the stomach of mammals as compared to the relatively neutral pH in the intestine of grass carp where the Zn complex may be more stable (Day et al. 2014). Stability of the dithiocarbamates is increased by the presence of the metal chelate which is stable under environmental conditions such as natural pH conditions (Wang et al. 2020; Weissmahr and Sedlak 2000).

In this study we found that acute toxicity of Ziram to freshwater fish ranges from 0.008 to 4 mg/L through aqueous exposure. No studies reported the aqueous toxicity of Ziram to grass carp. It should also be noted that most studies identified in this literature review did not measure Ziram in the exposure media. Additional water-only toxicity tests meeting data quality requirements, such as confirmation of exposure concentration, are

needed to confirm the sensitivity of non-target species to Ziram. Carp do not appear to have a species-specific sensitivity to Ziram in aqueous exposure. Carp species have sensitivities near or above the 50th percentile. Therefore, an approach of broad application of the chemical to a pond or lake to control carp is likely to affect non-target species. Additional efforts are needed to develop a method for selective delivery of Ziram for controlling grass carp while minimizing biological effects in non-target species.

A series of gavage experiments indicate the acute effect of Ziram, as prepared in these three carriers, was limited and nontoxic via the oral route. Future development of a piscicide bait using Ziram could consider methods to increase the absorption of Ziram in the intestine. Increasing residence time in the intestine may improve uptake. The bait formulation may need to incorporate ingredients, such as fats, that slow GI motility and increase residence time. Other alternatives include using different formulations of Ziram using different metal complexes, such as sodium or copper, that may have different physiochemical properties and dissociation in the intestine. This could allow for more of the chemical to be absorbed through the GI tract before it is eliminated by the fish.

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Authors' contributions

JAS conceived the project and secured and managed research ethics approval; NEK, KWG, DWW and JAS led sample design and methodology with feedback from DMW and MJH; NEK, KWG and DWW led implementation of investigations with support of JAS and DMW; NEK, and JAS conducted data analysisand interpretation with support of DMW and MJH; all authors contributed to the original drafting of the manuscript led by NEK, NEK and JAS led all authors as they reviewed and edited manuscript.

Ethics statement

All research presented here complies with requirements of the USGS-CERC Institutional Animal Care and Use Committee (IACUC), with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR), and with all CERC standard operating procedures for the humane treatment of test organisms during culture and experimentation. Experiments were designed to minimize the number of animals used and procedures used to avoid or minimize discomfort, distress, or pain to animals.

Data availability

Full data sets for the present study are available through the Supplemental Data and through a data repository (Kemble et al. 2022). Data, associated metadata, and calculation tools are also available from the corresponding author (nkemble@usgs.gov).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Chemical properties of Ziram.
Table S2. Output from Causal Analysis/Diagnosis Decisions Information System (CADDIS). Volume 4: Data Analysis Species Sensitivity Distribution Calculator.
Table S3. Results of three toxicity experiments testing the effects of Ziram administered by gavage or intraperitoneal (IP) injection.

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