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DRC-1339 AND DRC-2698 RESIDUES IN STARLINGS: PRELIMINARY EVALUATION OF THEIR EFFECTS ON SECONDARY HAZARD POTENTIAL

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INTRODUCTION

DRC-1339 (3-chloro-4-methylbenzenamine HCl) is the active ingredient in Starlicide Complete, a commercial bait used to control starlings (*Sturnus vulgaris*) at animal feedlots throughout the U.S. Because of the recent widespread use of this product, particularly within the wintering range of many raptors, they and other avian or mammalian scavenger or predator species may be exposed to large numbers of dead or dying starlings and blackbirds *(Icteridae)* throughout the winter roosting season (November-March). Acute toxicity data are available for five species of raptors and a number of mammals indicating that DRC-1339 or its primary toxic metabolite DRC-2698 (N-(3-chloro-4-methylphenyl) acetamide, CAT), a potential roost toxicant, are only moderately toxic (100-300 mg/kg) to these animals. However, there are some avian and mammalian scavengers or predators to which these compounds are considerably more toxic (i.e., cats, owls, magpies). Secondary hazards have not been observed when DRC-1339 killed starlings were fed to three raptor species for as long as 141 days and to domestic cats for seven days (DeCino, Cunningham and Schafer 1966; Holler et al. 1979, unpubl, manuscript); however, the direct long-term effects of both chemicals have not been determined on raptors or other predatory or scavenger species.

In order to decide if long-term studies are needed on predator or scavenger species, it is necessary to estimate accurately the degree of expected exposure to DRC-1339 and DRC-2698under actual use conditions. Since exposure will depend primarily upon the amount of these chemicals that remains in bird carcasses from bait ingestion until death, the metabolic or excretion rate of DRC-1339 and DRC-2698 must be known in target and at-risk, nontarget bird species. Although considerable data are available to determine the metabolic or excretion rate of DRC-1339 in target birds, some of these data are conflicting. We are therefore presenting the preliminary results of some of our current research with DRC-1339 and DRC-2698.

METHODS

Adult and subadult starlings were captured in the South Platte River Valley, Colorado, and held in indoor aviary facilities for a minimum of two months prior to testing. Only starlings with body weights greater that 70 g were tested. Technical grade DRC-1339 (>98%) was used for all studies, either incorporated in laboratory-prepared poultry mash or pellets or in propylene glycol solutions.

To determine carcass residues of DRC-1339 and DRC-2698 in starlings stomach-tubed with different levels of DRC-1339, 58 birds were food deprived for one hour and separated into 29 groups of two birds each, including controls. Birds in seven groups were stomach-tubed with propylene glycol solutions of DRC-1339 to administer dosages of 3.16, 10.0, 31.6 and 100 mg/kg; and one group was dosed with propylene glycol only Two birds from each dosage level were sacrificed at 10-minute intervals, quick frozen in

a methyl alcohol-dry ice solution, and held frozen (-17°C) in plastic bags until the carcasses were prepared for extraction analysis.

Residues of DRC-1339 and DRC-2698 were determined in the carcass of starlings fed starlicide pellets by dosing five groups of four randomly selected starlings (food deprived for 1 hour) at one of five treatment levels: 0, 3.16, 10, 31.6, and 100 mg/kg. Birds were force-fed single or multiple pellets to equal the desired dosage, individually caged, and observed until death or for seven days. All cages were equipped with removable stainless steel pans to collect fecal material. Survivors were killed with CO_2; carcass weight was recorded, the carcasses skinned, and the beak and legs removed. Fecal material and starling carcasses were placed separately in plastic bags, frozen, and held for extraction and analysis.

To extract DRC-1339, skinned, frozen starling carcasses were cut up in a Waring Blender for 3-5 minutes; and 5 g of tissue or 1.5 g of feces were homogenized at 20,000 RPM for 3 minutes in 30 ml of methanol, 16 g of sodium sulfate, and 1 ml of 1N hydrochloric acid. The homogenate was vacuum filtered, using a Buchner funnel and Whatman no. 1 filter paper, and rinsed with 20 ml methanol. Twenty ml of water plus 1 ml 1N hydrochloric acid were added to the filtrate, and the methanol was removed using a Buchler rotating vacuum evaporator. The resulting aqueous solution was agitated with 40 ml of hexane for 5 minutes to remove lipids. After partitioning, the hexane phase was discarded, and the hexane extraction repeated. After the second partition, the aqueous phase was made basic (pH 10.5) by adding 6 ml of 1N potassium carbonate and agitated with 40 ml of hexane for 10 minutes to recover DRC-1339 as the free-base (3-chloro-4-methylbenzenamine) in the hexane phase. The hexane phase was collected after partitioning and concentrated to a working volume of 2 ml.

To extract DRC-2698, 5 grams of tissue or 1.5 g of feces were homogenized at 20,000 RPM for 3 minutes with 30 ml methanol and 16 g of sodium sulfate. The homogenate was suction filtered, using a Buchner funnel and Whatman no. 1 filter paper, and rinsed with 20 ml of methanol. The filtrate was concentrated to 5 ml using the Buchler evaporator, 10 ml of water was added, and the solution was agitated for two minutes. Five ml of benzene was added to the water-methanol solution and agitated for 10 minutes. The benzene-methanol water mixture was centrifuged for six minutes at 4,000 RPM, the solution partitioned, and aliquots of the benzene supernatent injected into the gas chromatograph.

An MT-220 gas chromatograph equipped with a Tractor 702 thermionic NPD detector was used with a 0.9 m x 0.64 cm (o.d.) glass tube packed with 3% OV-17 on Gas Chrom Q, 80-100 mesh. The following instrument parameters were used for the analysis of tissue extracts for DRC-1339 and DRC-2698 residues.

a. DRC-1339: Temperature; column 200°C, inlet 245°C, detector 240°C. Flow rates; column (Helium) 60cc/min, detector (hydrogen) 2.0cc/min. Detector desensitiza - tion; 2.8 sec (automatic timed).
b. DRC-2698: Temperature; column 200°C, inlet 245°C. Flow rates; column (Helium) 70 cc/min, detector (hydrogen) 2.0cc/min. Detector desensitization; 2.8 sec (automatic timed).

Reference standards (0.1µg/µl) were prepared by dissolving 10 mg DRC-1339 in 100 ml of hexane or 10 mg of DRC-2689 in 100 ml of benzene. Injections of 0.1µg of DRC-1339 or DRC-2689 standards were made 3 or 4 times to attain a stable peak height response prior to each analysis. Responses in peak heights were linear from 0.001 to 0.01µg.

One to 5µl of samples were injected into the gas chromatograph, and the resultant peak height responses were compared to peak heights of 1-5µl injections of standards containing concentrations of 0.005-0.10µg/µl. Preliminary analysis indicated a 60% recovery of DRC-1339 and 47% of DRC-2698, with a limit of detectability of about 0.1 ppm.

RESULTS AND DISCUSSION

The data obtained to date are presented in Tables 1 and 2. Although they are still incomplete, a number of observations can be made that relate to the potential of DRC-1339-killed starlings presenting a secondary hazard to avian and mammalian predators and scavengers.

The stomach tube data (Table 1) were intended to further elaborate on the results of previous reports where the metabolic rate of DRC-1339 in starlings was estimated over a 5-7 hour period following dosage with 1.0 to 1.2 mg of DRC-1339. Peoples and Henry (1964) determined that starlings dosed orally with 1.0 mg of DRC-1339 excreted less than 10% as DRC-1339 and that less than 10% of DRC-1339 remained in the bird at death. They concluded that the remaining 80% was excreted in the feces in the form of two, non-toxic metabolites, 4-acetamino-2-chlorobenzoic acid (CPT-C) and 4-amino-2-cholorbenzoic acid (CPT-D). In 1965, Peoples indicated that starlings excreted most of a 1.0 mg DRC-1339 dose in two hours, as DRC-1339 and CPT-C. The total amount recovered after seven hours averaged from 66 to 100% of the amount administered. 15% as DRC-1339 and 51-85% as CPT-C. In 1967, Peoples and Apostolou reported that starlings dosed with 1.2 mg DRC-1339 excreted all of the dose administered within 4½ hours, 34.5% as DRC-1339 and 3.0% as DRC-2698 in the first 1½ hours. In 1969, Apostolou reported that during a 5-hour period following treatment only 20%of DRC-1339, DRC-2698, CPT-C, and CPT-D could be recovered from the fecal material of starlings dosed with 1.2 mg of DRC-1339. Forty-one percent of the excreted material was DRC-1339, 90% of which was excreted during the first hour. Chromatographic analysis (thin layer) of fecal extracts indicated that two additional unidentified metabolites also were present.

Although People's and Apostolou's results indicated that DRC-1339 was rapidly excreted and metabolized, a study by Westberg in 1969 showed that DRC-1339, DRC-2698, CPT-C, CPT-D residues were concentrated in the liver and kidneys but not brain or muscle following a 15 mg/kg dose of DRC-1339 in chickens. Peak tissue concentrations occurred approximately 30 minutes after treatment. Giri, Gribble, and Peoples (1976) reported that radio-labeled DRC-1339 administered to starlings intravenously (14.7µCi) was found to be unevenly distributed throughout the body. The half-life of radioactivity ranged from 3-6 hours in brain, spleen, heart, and bone marrow to 8-14.6 hours in muscle, lung, liver, and kidney.

In our study, less than 10% of a 100 mg/kg dose of DRC-1339 in starlings remained in the form of DRC-1339 or DRC-2698 30 minutes after treatment, indicating that more than 90% of the DRC-1339 administered had either been excreted or metabolized. Results with lower dosage levels indicated that, although the administered dose of DRC-1339 was initially metabolized or excreted very rapidly, from 1-5 ppm of DRC-1339 or DRC-2698 remained in body tissue throughout the entire 1-hour test period, regardless of the dosage administered. Thus, when starlings ingest amounts of DRC-1339 that are very close to the LD_{50} (3.76 mg/kg), less than 50% of the compound is excreted or metabolized during the first hour. This indicates limited, but rapid, binding to tissue, body protein, fat, or other body constituents.

When starlings were dosed with DRC-1339-treated pellets and their carcasses analyzed after they expired from DRC-1339 intoxication, the results (Table 2) were similar to the previous study. Starlings dosed with 3.16 to 100 mg/kg DRC-1339 all retained approximately 1 to 2 ppm of DRC-1339 and DRC-2698 residues at death. There was no obvious effect of dosage levels nor time to death on residue levels, indicating that small amounts of DRC-1339 and DRC-2698 are bound to some body component. Tests are underway to increase sample sizes for the stomach tube and pellet tests and to include fecal analyses; however, the results probably will not change from what we have reported, although variation between dosage levels should be reduced.

These data indicate that predator or scavenger birds or mammals subsisting wholly on DRC-1339-killed starlings are being exposed to a continuous 1-2 ppm level of DRC-1339 in their diet. For most predator or scavenger species, these residue levels do not

pose any hazard, since DRC-1339 has a low acute toxicity and negligible chronic toxicity to these species. Some species, however, may be at risk, particularly owls, cats, and magpies, since these species are all very sensitive to DRC-1339 intoxication. Although the possibilities of acute intoxication are negligible, since each animal would have to consume 2-3 times its own body weight at one feeding to ingest an acutely toxic dose, the possibility of chronic intoxication exists. Schafer, et al. (1977) have shown that DRC-1339 is a chronic toxicant in sensitive bird species, and there is no reason to believe that cats, owls, or magpies will be any different in this respect than the species they tested. Starlings ingesting 5.0 ppm DRC-1339 in their diet survived for an average of 27 days; 50 days at 2.5 ppm, and 77 days at 1.0 ppm; and there was no indication that the lower limits of chronic intoxication had been reached. Thus, DRC-1339-killed starlings may present a potential secondary hazard to cats, owls, and magpies, if their diet consists primarily of DRC-1339-killed starlings for more than 30 days. Because of these data, we feel that it may be useful to conduct limited chronic feeding studies (30 to 90 days) in cats and magpies, incorporating 1 to 2 ppm of DRC-1339 in their daily ration, or by feeding them DRC-1339-killed starlings for the same period of time.

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DISCUSSION

Q: Why is nobody pursuing this line of research for pigeon control?

A: I guess the biggest reason, at least why the Fish and Wildlife Service is not pursuing It, Is twofold. First, we are operationally not to undertake any work in urban situations. Because of that, research has not been oriented toward urban problems. I know the second reason is because there has been no outcry from people requesting this type of information. If PCOs or other people are interested In having us generate this type of Information, I think we need to know about it.

Q: Do you have any hypotheses on why the owls are affected? Do you think this could be a selective control technique?

A: Could very well be. I know for sure it could be a selective problem where you have cats that you want to get rid of. In fact, we've had some inquiry from people where they've had a problem with domestic cats and nesting birds.

Q: Is there any specific mechanism in owls that causes this problem?

A: I really don't know. There has been some information that would relate it to the presence or absence of certain enzyme systems in kidney tissue in different species. There are some data which refute some of the information that is published, but I don't think anyone really knows for sure.

TABLE 1. Skinned carcass residues of DRC-1339 and CAT from starlings gavaged with DRC-1339 solutions (n= 2).

Dosage level (mg/kg)	TIME Sacrificed (Min)	Carcass Residues (ppm)			
		DRC-1339	DRC-2598	Total	%Original dose remaining
100	0	60.2	3.4	63.6	63.6
	10	34.1	4.1	38.2	38.2
	20	23.9	2.4	26.3	26.3
	30	4.9	2.6	7.5	7.50
	40	2.9	1.3	4.2	4.20
	50	2.8	1.3	4.1	4.10
	60	4.2	0.55	4.75	4.75
31.6	0	4.79	1.23	6.02	19.0
	10	4.8	1.3	6.1	19.3
	20	4.8	0.8	5.6	17.7
	30	2.9	0.4	3.3	10.4
	40	4.3	1.0	5.3	16.8
	50	4.8	1.5	6.3	19.9
	60	2.7	1.2	3.9	12.3
10.0	0	5.50	1.29	6.79	67.9
	10	4.1	0.8	4.9	49.0
	20	1.8	0.6	2.4	24.0
	30	1.2	0.6	1.8	18.0
	40	3.1	1.5	4.6	46.0
	50	0.6	0.5	0.6	6.00
	60	0.9	0.7	1.6	23.0
3.16	0	3.3	1.0	4.3	136
	10	1.1	0.5	1.6	50.6
	20	1.8	1.0	2.8	88.6
	30	1.3	0.5	1.3	41.1
	40	1.1	0.5	1.1	34.8
	50	1.1	0.5	1.1	34.8
	60	2.6	1.0	3.6	114

TABLE 2. Skinned-carcass residues at death of DRC-1339 and CAT from starlings fed 1% DRC-1339-treated pellets.

Dosage Level (mg/kg)	Bird No.	Death Time (hrs.)	Carcass Residues (ppm) DRC-1339	% Original DRC-2696	Dose remaining
100	1	>4 <16	2.2	<0.25	2.2
	2	>4 <16	2.2	<0.25	2.2
	3	>4 <16	0.1	<0.25	0.1
	4	>4 <16	0.2	<0.25	0.2
	AVERAGE	>4 <16	1.2	<0.25	1.2
31.6	1	>32 <47	0.2	<0.25	0.5
	2	>32 <47	0.9	<0.25	2.6
	3	>32 <47	1.1	<0.25	3.5
	4	>32 <47	0.1	<0.25	0.3
	AVERAGE	>32 <47	0.6	<0.25	1.9
10.0	1	69	0.5	<0.25	5.0
	2	21	1.1	<0.25	11.0
	3	120	2.8	<0.25	28.0
	4	60	4.3	<0.25	43.0
	AVERAGE		2.2	<0.25	22.0
3.16	1	...	...	...	...
	2	47	0.2	<0.25	6.3
	3	47	0.1	<0.25	3.2
	4	>94 <99	0.05	<0.25	<1.6
	AVERAGE		0.1	<0.25	<3.7