NONTARGET BIRD EXPOSURE TO DRC-1339 DURING FALL IN NORTH DAKOTA AND SPRING IN SOUTH DAKOTA

THOMAS W. CUSTER, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, LaCrosse, WI 54603, USA

CHRISTINE M. CUSTER, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, LaCrosse, WI 54603, USA

PAUL M. DUMMER, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, LaCrosse, WI 54603, USA

GEORGE M. LINZ, U.S. Department of Agriculture, National Wildlife Research Center, Great Plains Field Station, 2110 Miriam Circle, Bismarck, ND 58501, USA

LOUIS SILEO, U.S. Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711, USA

RANDAL S. STAHL, U.S. Department of Agriculture, National Wildlife Research Center, Analytical Chemistry Project, 4101 LaPorte Avenue, Fort Collins, CO 80521-2154, USA

JOHN J. JOHNSTON, U.S. Department of Agriculture, National Wildlife Research Center, Analytical Chemistry Project, 4101 LaPorte Avenue, Fort Collins, CO 80521-2154, USA

Abstract: Blackbirds frequently use ripening sunflower (*Helianthus annuus*) as a food source in the northern Great Plains. In 1999 and 2000, the avicide DRC-1339 (3-chloro-4-methylaniline hydrochloride) was used experimentally on fall-ripening sunflower fields in North Dakota so researchers could evaluate its effectiveness for reducing crop depredations by blackbirds. DRC-1339 was applied to rice and broadcast on the ground in a confined area within ripening sunflower fields. One objective of this study was to determine whether nontarget birds, birds other than blackbirds, were eating rice and were exposed to the DRC-1339. In 1999, 8 of 11 (73%) sparrows collected by shotgun in sunflower fields treated with DRC-1339 had rice in their gastrointestinal (GI) tracts. In 2000, 5 mourning doves (*Zenaida macroura*) and 3 sparrows were collected by shotgun in sunflower fields treated with DRC-1339. Three doves had rice in their GI tracts, 4 doves and all 3 sparrows had measurable DRC-1339 concentrations in their GI tracts, and 3 mourning doves and 1 savannah sparrow (*Passerculus sandwichensis*) exhibited histopathological signs of kidney damage. In April 2002, untreated rice was applied to corn stubble plots in South Dakota to determine which bird species ate rice. In 2002, 3 of 3 song sparrows (*Melospiza melodia*) collected by shotgun had rice in their GI tracts. Our results demonstrate that the use of DRC-1339 to control blackbirds in the northern Great Plains will likely expose nontarget birds to the DRC-1339 bait.

Key words: avicides, blackbirds, DRC-1339, mourning doves, sparrows, sunflowers.

Blackbirds and grackles forage on ripening sunflower (*Helianthus annuus*) crops in the northern Great Plains (Linz and Hanzel 1997). In 1997, 47% of North Dakota sunflower growers reported damage > 5%; 26% reported damage 5-10%, 15% reported 10-25% damage and 6% reported damage 25% to 100%. Similar results were reported for South Dakota (Lamey et al. 1999).

DRC-1339 (3-chloro-4-methylaniline hydrochloride), an avicide developed by the U.S. Fish and Wildlife Service for controlling European starlings (*Sturnus vulgaris*) at feedlots (DeCino et al. 1966), has also been used to reduce blackbird populations responsible for damaging sprouting rice in Louisiana (Glahn and Wilson 1992). The U.S. Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) evaluated the effectiveness of DRC-1339 for its ability to reduce blackbird damage in the fall to ripening sunflower (Linz and Bergman 1996) and to reduce blackbird breeding populations in South Dakota (Linz et al. 2002). The

spring applications are an attempt to reduce blackbird numbers that could potentially damage ripening sunflower in North Dakota and South Dakota.

DRC-1339 poisoning in European starlings was characterized by a white, fat-like material that collected within the body cavity, particularly in the pericardial region (DeCino et al. 1966). Depending on the time since exposure, DRC-1339 causes microscopic cloudy swelling, hydropic degeneration, nuclear chromatolysis and coagulation necrosis of the proximal tubules of the kidney. (DeCino et al. 1966, Peoples and Apostolou 1966). A method to quantify DRC-1339 residues in bird carcasses was developed and validated using laboratory DRC-1339 fortified tissues (Hurlbut et al. 1998, Stahl et al. 2002) and tissues from boat-tailed grackles (*Quiscalus major*) that ate a wide range of lethal doses of DRC-1339 (Johnston et al. 1999).

Laboratory studies suggest that birds that eat DRC-1339-treated bait could be negatively affected (USDA 1994). Nontarget birds have been observed in fall sunflower fields baited with DRC-1339 (Schaaf et al. 2001) and in spring fields near blackbird roosts (Linz et al. 1995, Linz et al. 2002); however, it was unknown if they were eating rice and significant quantities of DRC-1339. To enhance our knowledge about the toxic risks of DRC-1339 to nontarget species, Linz et al. (2002) recommended dietary analyses of species feeding in baited areas.

One objective of this study was to evaluate DRC-1339 related exposure to nontarget birds feeding in DRC-1339 fall-baited sunflower fields in North Dakota. First, we wanted to establish whether nontarget birds were eating rice in sunflower fields baited with DRC-1339. If nontarget birds were eating DRC-1339 bait, we then wanted to determine if DRC-1339 residues could be detected in their tissues, determine whether the pericardial cavity had white crystals indicative of DRC-1339 poisoning, and determine if there was any evidence of kidney damage. Another objective of this study was to determine whether nontarget birds observed in spring-baited plots were eating rice.

METHODS

Fall Sunflower Fields, North Dakota

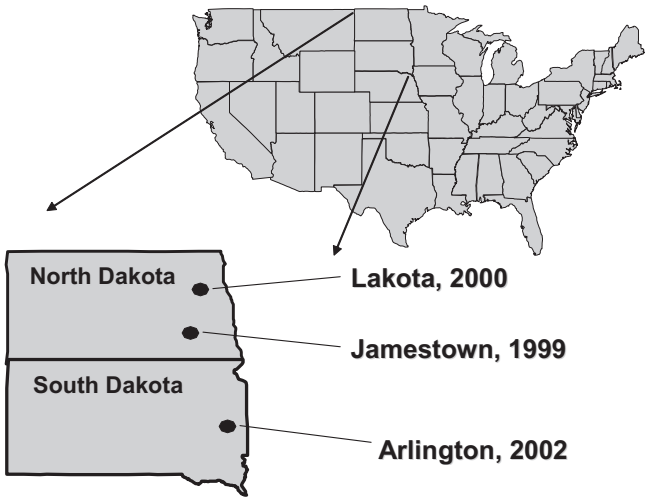
Fields in North Dakota in 1999 and 2000 were selected and treated with DRC-1339 as follows. Following a request from a sunflower grower, the field was evaluated by USDA Wildlife Services personnel for damage by blackbirds. If bird damage was evident, a four-wheel drive all-terrain vehicle pushed sunflowers to the ground in a 0.1 ha plot in the sunflower field and 2.8 kg of untreated brown rice was applied to the plot. When 50% or more of the untreated rice was eaten, the site was treated with 0.11 kg of DRC-1339 rice baits (2% concentration, 0.4 mg/grain) and 2.8 kg lbs of untreated rice. The treated and untreated rice were gently mixed before transferring the rice mixture into a spreader and distributing evenly over the plot.

During 1-3 September 1999, sunflower fields treated with DRC-1339 were observed in Barnes and Stutsman counties near Jamestown, North Dakota (Fig. 1). Usually, 2 observers approached treated areas and if birds were present in the plot, they were collected using a shotgun (lead shot, sizes ranged from 7.5–9). If the site appeared to be active with nontarget birds, 1 observer hid in the sunflower field and collected nontarget birds as they came to the area. Once birds were collected, the esophagus and stomach (upper GI tract) were removed and the contents of each were washed separately into jars filled with alcohol. The contents of each esophagus and stomach were later categorized as to whether they contained rice, other seeds, or arthropods.

During 13-18 September 2000, 2 sunflower fields treated with DRC-1339 were observed in Nelson County near Lakota, North Dakota (Fig. 1). Within 24 hr after treatment with DRC-1339, an observer was positioned in a blind at the edge of the treated area and continuous daylight observations were conducted for approximately 30 hrs at each field. Once a bird was collected, the upper GI tract was removed, put into a labeled chemically-clean jar and placed on wet ice until later frozen. The kidneys were removed and placed in a labeled jar filled with 10% buffered neutral formalin and notations were made regarding the presence or absence of a white residue in the body cavity, particularly the pericardial region. The carcass remainder was labeled, wrapped in aluminum foil, sealed in a plastic bag, placed on wet ice within one hour of death and later frozen. The upper GI tract contents were identified and the number of rice grains estimated based on whole rice and pieces of rice. The preserved tissues were submitted to the histology laboratory of the College of Veterinary Medicine, University of Wisconsin, Madison, for preparation of microscope slides. Sections of the kidney samples were stained by the hematoxylin and eosin method and examined by light microscope to determine if there was evidence of kidney damage.

For the birds collected in the year 2000, a sample of breast muscle tissue and the upper GI tract including contents were analyzed for DRC-1339 using the methods described by Johnston et al. (1999) and Stahl et al. (2002). The limits of detection for samples on 4 separate days were 0.0065, 0.019, 0.038, and 0.039 µg/g wet weight. Sample results were not adjusted for sample recovery, which averaged 93%. All birds were collected in 1999 and 2000 under appropriate state and federal collecting permits.

Fig. 1. Study sites in North Dakota (1999 and 2000) and South Dakota (2002).



Spring Corn Stubble Fields, South Dakota

From 7-12 April 2002, 3 harvested cornfields ≤5 km from 4 blackbird roosts were observed in Brookings County near Arlington, South Dakota (Fig. 1). Each field contained 2 baited 0.4-ha plots. The baited plots where located adjacent to one another in all but 1 instance. In that instance, the plots where in the same field but separated by about 350 m. In the baited fields, an all-terrain vehicle was used to broadcast 23 kg of brown rice per plot acre. A portable blind was erected in the center of 1 of the 2 plots in each field. To habituate birds to the presence of the blinds, a blind was left in 1 of the plots in each field for the duration of the study.

Observations occurred during daylight hours from dawn to dusk and were divided into 4-6 hr shifts. During a shift, an observer concealed within a blind, recorded bird activity in the plot and at the edge of the plot.

Observers attempted to collect all nontarget birds and a sample of blackbirds observed feeding in the plot by shotgun (size 7½-9 lead shot). For each specimen, we recorded the bird's common name, collection time and location. The upper GI tract was removed, weighed and placed into alcohol. Carcasses where kept under refrigeration until transferred to a freezer at the Upper Midwest Environmental Sciences Center (UMESC). At UMESC, the upper GI tract was opened and the number of rice grains in the esophagus and stomach was estimated based on whole rice and parts of rice present. To our knowledge, the only source of rice available to birds collected during the studies in North Dakota (1999, 2000) and South Dakota (2002) was that applied to the fields as part of this study.

Table 1. Number of rice grains present in the upper GI tract of nontarget (nonblackbird) birds collected in ripening sunflower fields during 1-3 September 1999.

	No. of rice grains present	
Species	Esophagus	Stomach
Savannah sparrow	2	2
Savannah sparrow	1	2
Savannah sparrow	1	1
Savannah sparrow	0	-[a]
Savannah sparrow	0	-
Vesper sparrow	0	2
Vesper sparrow	1	2
Vesper sparrow	1	1
Vesper sparrow	0	-
Grasshopper sparrow	0	2
Grasshopper sparrow	0	1
Rock dove	-	-
Rock dove	-	-
Mourning dove	-	-

[a]indicates food items other than rice

RESULTS

Fall Sunflower Fields, North Dakota

In 1999, 5 savannah sparrows (*Passerculus sandwichensis*), 4 vesper sparrows (*Pooecetes gramineus*), 2 grasshopper sparrows (*Ammodramus savannarum*), 2 rock doves (*Columba livia*), and 1 mourning dove (*Zenaida macroura*) were collected in the DRC-1339-treated areas. Eight of the 11 sparrows (73%) had rice in the esophagus or stomach (Table 1). Two rock doves and 1 mourning dove did not have rice in the esophagus or stomach, but all did contain other types of seeds, such as sunflower, canola, and wheat.

In 2000, 2 fields near Lakota, North Dakota were baited with DRC-1339. During 60 hrs of daylight observation at both sites combined, 12 mourning doves, 18 sparrows, one American robin (*Turdus migratorius*), and 350 blackbirds were observed on the plots (Table 2). Four mourning doves, 18 sparrows, 3 robins, and 500 blackbirds were observed in the sunflowers at the edge of the plot. About 1 granivorous nontarget bird was seen on the plots for every 2 hrs of observation.

Table 2. Number of nontarget birds and blackbirds counted during 60 hrs of observation in ripening sunflower fields treated with DRC-1339 during 13-18 September 2000 in North Dakota.

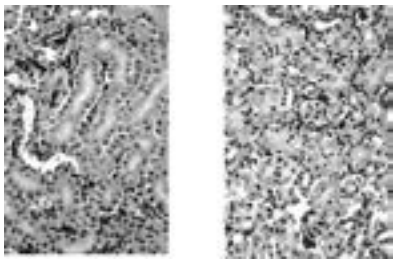
	Number birds in plots	Number birds in plot/hr	Number birds at edge of plot	Total number birds/hr
Mourning doves	12	0.2	4	0.27
Sparrows	18	0.3	18	0.60
Robins	1	0.02	3	0.07
Total nontargets	31	0.52	25	0.93
Blackbirds	350	5.8	500	14.2

Table 3. Chemical concentration, histopathology, and GI tract contents in 5 mourning doves and 3 sparrows collected near Lakota, North Dakota, during 15-18 September 2000. Birds were collected in ripening sunflower fields following application of DRC-1339-treated-rice. Multiple DRC-1339 entries indicate duplicate samples.

Species/No.	Tissue	Concentration of DRC-1339 mg/g wet weight	Kidney diagnosis	Contents of GI tract
M. dove-1	GI tract Carcass	0.078, 0.102 0.009, 0.013	severe nephrosis	55+ rice in esophagus 2 rice in stomach
M. dove-2	GI tract Carcass	0.103, 0.095, 0.091, 0.07 nd-a[a], nd-a	none	134 rice in esophagus 2 rice in stomach
M. dove-3	GI tract Carcass	nd-b, nd-b 0.008, nd-a	none	no rice
M. dove-4	GI tract Carcass	1.45, 0.78 0.014, nd-a	minor nephrosis	19 rice in esophagus 3 rice in stomach
M. dove-5	GI tract Carcass	nd-b, nd-b nd-b, nd-b	minor nephritis	no rice
Vesper-6	GI tract Carcass	0.052 nd-a, nd-a	none	no rice
Grasshop-7	GI tract Carcass	0.271 nd-a	none	no rice
Savannah-8	GI tract Carcass	0.08, 0.02 nd-a	renal vacuolar degeneration	no rice

[a]nd-a = not detected at 0.0065 µg/g wet weight; nd-b = not detected at 0.039 µg/g wet weight

Five mourning doves, 1 savannah sparrow, 1 grasshopper sparrow, and 1 vesper sparrow were collected near Lakota, North Dakota between 15-18 September 2000. DRC-1339 was detected in the carcass or upper GI tract of all 3 sparrows and 4 of 5 mourning doves (Table 3). The maximum concentration of DRC-1339 in the GI tracts and carcasses was 1.45 µg/g and 0.014 µg/g, respectively, recorded in a mourning dove. Three of 5 mourning doves had rice in the GI tract; none of the sparrows had rice in their GI tracts. No white crystals were observed around the heart or liver in any of the birds collected.



No diagnostic lesions Severe Nephrosis

Fig. 2. Mourning dove kidney tissues with no diagnostic lesions (left) and severe nephrosis (right; No. 1, Table 3). Separation of the proximal epithelial cells is an autolytic artefact in the left section. Severe nephrosis affects the entire right section of tissue. Proximal epithelial cells are distorted and indistinct; many lack nuclei. Some cells have basophilic cytoplasm. Some nuclei lack nucleoli and have marginated chromatin. Some nuclei are slightly enlarged. Sloughed necrotic cells and eosinophilic debris occlude the lumens of some tubules.

Microscopic examination revealed unequivocal abnormalities (vacuolar degeneration, nephrosis, and nephritis) in the kidneys of 4 of the birds (Table 3). The severity of the lesions ranged from minimal to severe. The nephrosis in the most severely affected mourning dove was extensive, involving the entire section of tissue, and was characterized by distorted, indistinct proximal epithelial cells that lacked nuclei with slightly basophilic cytoplasm (Fig. 2). Many nuclei lacked nucleoli and had marginated chromatin; some nuclei were slightly enlarged. Sloughed necrotic cells and eosinophilic debris occluded the lumens of many tubules. The lumens of a few tubules contained mineralized debris associated with flattened epithelial cells. An occasional granulocyte could be seen amongst the damaged epithelial cells. A few regenerating tubules were lined with hypertrophied, basophilic cells and a few subcapsular and interstitial aggregates of lymphocytes were present. Nephrosis of this severity and extent would have been life-threatening. The nephritis (No. 5, mourning dove), which was minimal to mild in severity, was of the chronic interstitial type characterized by aggregates of lymphocytes in subcapsular or interstitial locations and sometimes associated with degenerate or necrotic tubules. The renal vacuolation (No. 8, savannah sparrow) was characterized by multifocal distribution of proximal tubules with epithelial cells with cytoplasmic vacuoles that were round, smooth, and empty. This was a very mild abnormality.

The field where the dove with severe nephrosis was collected was baited 32 hrs prior to collection; the fields where the 2 doves with minor nephrosis (No. 4, Table 3) and minor nephritis (No.5) were collected,

Table 4. Number of nontarget birds and blackbirds counted during 128 hrs observation of corn stubble fields treated with rice in South Dakota in April 2002.

	No. in plot	No. birds in plot/hr	No. near plot	Total no. observed/ hr
Mourning dove *Zenaida macroura*	0	0	8	0.06
Sparrows	10	0.08	22	0.25
Western meadowlark *Sturnella neglecta*	1	<0.01	1	0.02
Dark-eyed junco *Junco hyemalis*	1	<0.01	2	0.02
Ring-necked pheasant *Phasianus colchicus*	4	0.03	14	0.14
Canada goose *Branta canadensis*	2	0.02	2	0.03
Black-capped chickadee *Parus atricapillus*	0	0	1	<0.01
American crow *Corvus brachyrhnchos*	1	<0.01	0	<0.01
Killdeer *Charadrius vociferus*	0	0	4	0.03
Northern flicker *Colaptes auratus*	1	<0.01	0	<0.01
Downey woodpecker *Picoides pubescens*	1	<0.01	0	<0.01
American Robin *Turdus migratorius*	48	0.38	21	0.54
Blue jay *Cyanocitta cristata*	0	0	1	<0.01
Total nontargets	69	0.54	66	1.05
Blackbirds	37,661	294	25,930	497

were baited 50 and 51 hrs prior to collection. The savannah sparrow (No. 8) was collected 65 hrs after the field was baited.

Spring Corn Stubble Fields, South Dakota

In 2002, 69 nontarget birds and about 37,000 blackbirds were observed on the plot during 128 hrs of observation (Table 4). Of the 69 nontarget birds, 18 were granivores. This equates to about 1 granivorous nontarget bird every 7 hrs of observation.

Three song sparrows (*Melospiza melodia*) were collected in the rice-baited areas in 2002. All 3 song sparrows had rice grains in the upper GI tract (Table 5). One northern flicker (*Colaptes auratus*) and 2 American robins did not have rice in the upper GI tract. One of the 5 common grackles (*Quiscalus quiscula*) had rice grains in the upper GI tract. The remaining 4 common grackle samples had no rice in the upper GI tract. All common grackle GI tracts contained food items, usually corn. Ten of 13 red-winged blackbirds (*Agelaius phoeniceus*) collected in the rice-baited plots had rice in the upper GI tract.

DISCUSSION

Our study confirms that free-living nontarget birds will eat rice when applied to ripening sunflower fields in North Dakota and corn stubble fields in South Dakota. A pen study demonstrated that female ring-necked pheasants (*Phasianus colchicus*) will eat rice even when given a choice of cracked corn and sorghum (Avery et al. 1998).

The low concentration of DRC-1339 in breast muscle relative (maximum 0.014 µg/g, Table 3) to that in the GI tract (maximum 1.45 µg/g) is consistent with earlier reports (Johnston et al. 1999, Stahl et al. 2002). DRC-1339 is quickly metabolized and excreted accounting for low residues (≤0.1 µg/g) in tissues of poisoned birds (Johnston et al. 1999). Breast residue in 5 of 21 birds poisoned with DRC-1339 did not have detectable concentrations of DRC-1339; the GI tracts of 7 of 21 also did not have detectable concentrations of DRC-1339 (Johnston et al. 1999). Four red-winged blackbirds and 4 brown-headed cowbirds (*Molothrus ater*) shot over treated bait had <0.15 µg/g DRC-1339 in their breast muscle and 2-44 µg/g in the GI tract (Stahl et al. 2002).

Table 5. Presence of rice in the upper GI tract of birds collected in fields treated with rice bait in South Dakota, 2002.

Species	Presence of rice[a] Esophagus	Stomach	Number of rice grains
Northern flicker	-	-	0
Song sparrow	+	+	4
Song sparrow	+	-	2
Song sparrow	+	+	5
American robin	-	-	0
American robin	-	-	0
Common grackle	-	-	0
Common grackle	-	-	0
Common grackle	+	+	5
Common grackle	-	-	0
Common grackle	*	-	0
Red-winged blackbird	*	+	2
Red-winged blackbird	+	+	6
Red-winged blackbird	-	-	0
Red-winged blackbird	+	+	13
Red-winged blackbird	+	+	36
Red-winged blackbird	-	-	0
Red-winged blackbird	+	-	1
Red-winged blackbird	-	+	2
Red-winged blackbird	-	-	0
Red-winged blackbird	-	+	1
Red-winged blackbird	+	+	4
Red-winged blackbird	-	+	1
Red-winged blackbird	+	+	5

[a] -= rice not present, + = rice present, * = sample destroyed during collection

Even though DRC-1339 residues were detected and kidney damage reported, the white residue in the pericardial cavity associated with DRC-1339 poisoning (DeCino et al. 1966) was not observed in this study. DeCino et al. (1966), however, examined birds that had succumbed to DRC-1339 poisoning. Our specimens were collected prior to death, presumably before pericardial deposits formed. Residues were also better predictor of DRC-1339 ingestion than necropsy in boat-tailed grackles poisoned with DRC-1339; of 21 grackles poisoned with DRC-1339, 17 had detectable residues and 11 had pericardial deposits (Johnston et al. 1999).

The maximum time of exposure to DRC-1339 for the birds that exhibited kidney damage (32 to 65 hrs) is consistent with earlier reports. Felsenstein et al. (1974) reported that within 24 to 72 hrs of ingestion highly susceptible birds exhibited irreversible kidney and heart damage causing death.

Given that mourning doves ate rice, exposure and effects of DRC-1339 to mourning dove were expected. Because 1 in 26 rice grains were treated with DRC-1339 and 3 mourning doves collected in 2000 had 19, >55, and 134 rice grains in their crop, there was a high probability that mourning doves would encounter a treated rice grain during a feeding bout. Additionally, mourning doves are sensitive to DRC-1339 poisoning (Eisemann et al. 2003). For example, consumption of 42 rice grains (2 treated grains) during a single feeding bout would be approximately equivalent to the mourning dove LD50 (calculation in Eisemann et al. 2001).

Sparrows feeding in the rice-baited plots are probably not as exposed to DRC-1339 as mourning doves and predicting the effects of DRC-1339 is more problematic. Because the sparrows ate fewer rice grains compared to mourning doves (Table 3), the odds of a sparrow encountering a treated rice grain were lower. The sensitivities of the vesper sparrow and grasshopper sparrow to DRC-1339 were also unknown. These 2 species are members of the family Emberizidae and the sensitivities of species within this family varies from highly sensitive to insensitive. The LD_{50} of the dark-eyed junco (*Junco hyemalis*) was 162 µg/g (Eisemann et al. 2003). In contrast, the LD_{50} for the American tree sparrows (*Spizella arborea*) was 3.5 µg/g (Eisemann et al. 2003). White-crowned sparrows (*Zonotrichia leucophrys*) were reported to have an LD_{50} value of >320 µg/g (Schafer et al. 1983). However, because this value is based on only 2 individuals (Eisemann et al. 2003), it must be viewed cautiously. Acute dietary toxicity studies suggest that song sparrows and savannah sparrows are insensitive to DRC-1339 (Eisemann et al. 2003). The LC_{50}s for these 2 species were estimated at >714 ppm.

It is very likely that the kidney lesions detected in nontarget birds were caused by exposure to DRC-1339. Nephrosis of kidney tissues, which is often an effect of blood-borne toxins (Brown 1996), was documented in nontarget birds. Additionally, DRC-1339 was detected in the GI tracts and carcasses of nontarget birds.

Even though earlier reports of the histopathology of DRC-1339 toxicity briefly described the renal lesion as ranging from "cloudy swelling to necrosis" (DeCino et al. 1966, Peoples and Apostolou 1966), the term nephrosis, (non-inflammatory necrosis of epithelial cells of the proximal tubules) better describes the salient lesion in the birds we examined. Renal vacuolar degeneration is an early degenerative process that might have preceded full blown nephrosis. Chronic interstitial nephritis might have been a reparative consequence of nephrosis.

The difference in sensitivity to DRC-1339 among members within Emberizidae (Eisemann et al. 2001, 2003) suggests that the classification of sensitivity of birds to DRC-1339 based on avian families may not be adequate. As such, a definitive risk evaluation of DRC-1339 to nontarget birds requires exposure assessments and dose-response toxicity assessments for species that eat rice during DRC-1339 baiting operations. We suggest that further toxicity testing should focus on vesper sparrows and grasshopper sparrows. Of all of the species identified in this study that ate rice, only these 2 species lack DRC-1339 toxicity assessments (Eisemann et al. 2003).

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