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OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: **Nicarbazin:** Draft Ecological Risk Assessment for Registration Review

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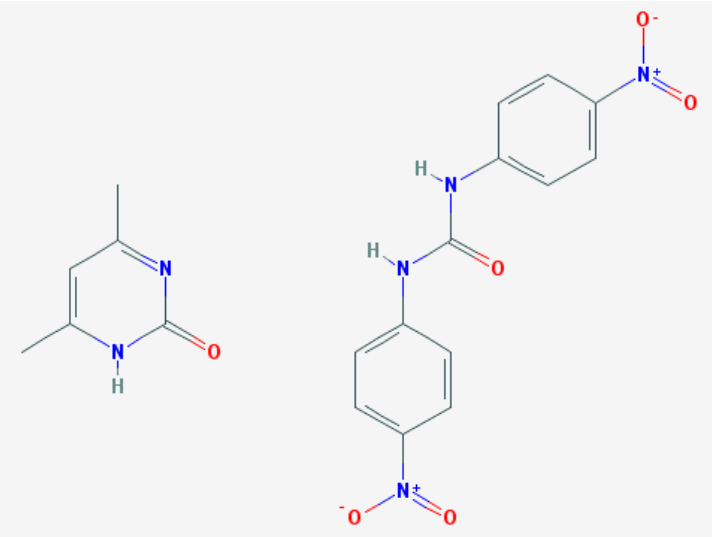
The Environmental Fate and Effects Division (EFED) has completed the draft environmental fate and ecological risk assessment in support of the Registration Review of nicarbazin, an avian contraceptive, formulated as a ready-to-use control agent (treated bait) for reducing egg viability and hatchability in pigeons (*Columba livia*, rock dove, feral pigeons), European starlings (*Sturnus vulgaris*), red-winged blackbirds (*Agelaius phoeniceus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), brewers blackbirds (*Euphagus cyanocephalus*), great-tailed grackles (*Quiscalus mexicanus*), boat-tailed grackles (*Quiscalus major*), common grackles (*Quiscalus quiscula*), brown-headed cowbirds (*Molothrus ater*), bronzed cowbirds (*Molothrus aeneus*), and the common myna (*Acridotheres tristis*).

Draft Ecological Risk Assessment for the Registration Review of Nicarbazin



Nicarbazin Complex (HDP (left) and DNC (right));
CAS No.: Nicarbazin complex 330-95-0; DNC 587-90-6 and HDP 108-79-2

USEPA PC Code: 085712

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1 Executive Summary

1.1 Overview

This document provides the draft risk assessment (DRA) for nicarbazin. Nicarbazin is a complex made up of two components in a 1:1 molar ratio, 4,4'-dinitrocarbanilide (DNC, 70.89% by weight) and 4,6-dimethyl-2-pyrimidinol (HDP, 29.11% by weight). DNC is considered to be the active component, and HDP aids in its absorption. The potency of DNC is reported to be increased tenfold when it is complexed with HDP (Chapman 1994). Nicarbazin (OvoControl P; EPA Reg. No: 80224-1) is currently registered for use as an avian contraceptive formulated as a ready-to-use control agent (treated bait) to reduce the viability and hatchability of eggs by interfering with the formation of the vitelline membrane, which separates the egg yolk and the egg white. Nicarbazin is registered for use on pigeons (*Columba livia*, rock dove, feral pigeons), European starlings (*Sturnus vulgaris*), red-winged blackbirds (*Agelaius phoeniceus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), brewers blackbirds (*Euphagus cyanocephalus*), great-tailed grackles (*Quiscalus mexicanus*), boat-tailed grackles (*Quiscalus major*), common grackles (*Quiscalus quiscula*), brown-headed cowbirds (*Molothrus ater*), bronzed cowbirds (*Molothrus aeneus*), and the common myna (*Acridotheres tristis*). Historically, nicarbazin has been used since the mid-1950s as an anticoccidial agent to control intestinal protozoan parasites in the poultry industry. There are no agricultural pesticidal uses for nicarbazin. It is for outdoor use only in non-food areas of manufacturing facilities, power utilities, hospitals, food processing plants, distribution centers, oil refineries and processing centers, chemical plants, rail yards, schools, campuses, military bases, seaports, hotels, apartments, condominiums, maintenance yards, shopping malls, feed mills, airports and other commercial, residential or industrial locations. OvoControl P may only be applied to rooftops or other flat paved or concrete surfaces in secured areas with limited public access or areas under direct supervision of the applicator.

1.2 Risk Conclusions Summary

This DRA examines the potential ecological risks associated with labeled uses of nicarbazin as a treated bait used to reduce viability and hatchability of eggs to non-target organisms not listed as Federally threatened or endangered species. The risk assessment uses a streamlined approach to focus on the taxa of primary risk concern based on previously completed risk assessments, and also taxa for which additional data have become available. Taxa of focus in this assessment include non-target birds and mammals. Other non-target plants and animals, including aquatic organisms (including aquatic plants), terrestrial plants and terrestrial invertebrates are not expected to be at risk from use of nicarbazin due to a lack of exposure or low likelihood of exposure.

Risk conclusions for birds and mammals are based on very limited guideline and literature data for both birds and mammals. In general, when used in accordance with the label, although

some of these statements may be viewed as more advisory rather than mandatory (in secured areas with limited public access or areas under direct supervision of the applicator, with excess bait removed and feeding halted if non-target birds and mammals present at time of application), it is likely that non-target wildlife exposures will be limited.

However, if non-target birds were to consume treated bait, nicarbazin may pose both an acute risk (RQ value, **1.4** (dietary-based)) and chronic risk (when comparing the estimated environmental concentrations (EECs) to concentrations in bait). Although small birds (20g) would have the potential to exceed the acute dose-based risk LOC of 0.5 based on an exposure/toxicity ratio value of <**0.77**, this was a conservative estimate of acute dose-based risk (as there were no effects noted at the highest-dose tested which was conservatively used to calculate an exposure/toxicity ratio). Additionally, based on the acute oral toxicity data available, birds must consume about 6.5g bait to reach the LD_{50} dose, which is >100% of a small bird's daily diet, (5g for 20g birds; according to the *Wildlife Exposure Factors Handbook* (USEPA, 1993)). However, it is noted that acute oral passerine data was not submitted, therefore there is uncertainty of the difference in sensitivity of passerines to non-passerine species, and therefore risk to passerines (and potentially small birds) cannot be precluded on an acute oral or dietary basis). There are no reported incidents, for birds or any taxa.

Birds fed OvoControl P according to label directions may still continue to lay eggs, , but this product will reduce egg hatchability and adversely affect other aspects of reproduction in all avian species, based on the mode of action of nicarbazin. Nicarbazin, a chemical that has been historically used over the years in the poultry industry in broiler chickens as food additive to control intestinal protozoan parasites is not used on layer hens due to the observed reproductive effects of the chemical.

The results of the recently submitted chicken reproduction study can only be used qualitatively to compare the nicarbazin concentration in bait versus the chronic endpoint (increased early embryonic mortality). Chickens are not a typical test species for evaluating chronic effects of pesticides to birds and there is additional uncertainty in extrapolating effects from this species across the taxa compared to the standard surrogate test species for which data are typically generated to evaluate avian toxicity. It is noted that the chicken reproduction endpoint is likely not as conservative compared to our standard avian reproduction endpoint for evaluating reproductive/chronic effects to non-target birds due to the study design and evaluated endpoints. The concentration in bait was 2500x when compared to a reproductive endpoint (increased early embryonic mortality) using chickens. In order to estimate the chronic dietary-based risk, both the during and post-exposure endpoints were compared for characterization of nicarbazin. This showed that when the nicarbazin bait is removed from the diet, two-weeks after exposure, statistically significant effects were not observed on any measured endpoint. Therefore, the resulting risk appears to decrease over time, although there was still risk when comparing the tested concentrations to concentrations in bait. The concentration in bait was 125x the post-exposure chicken reproductive endpoint as the No Observable Adverse Effects Concentrations (or NOAEC) was 40 mg/kg-diet, the highest dose tested. Given nicarbazin's mode of action and intended use as a bird reproduction inhibitor, this is not unexpected.

Nicarbazin has low acute mammalian toxicity. Similar to non-target birds, exposure to non-target mammals may be minimal if nicarbazin is used according to the label, however, if mammals are able to access nicarbazin bait at the site of application by accessing bait pellets placed on the ground/in bait pans there may be potential chronic risk (based on decreases in body weight at the Lowest Observable Adverse Effects Level {LOAEL}). The dose-based chronic risk estimates for mammals exceed the dose-based chronic risk LOC (1.0) for all sizes of mammals assessed, (RQ values range from **1.1** – **2.5**). Chronic risk to mammals could be even greater, as the RQs presented here are based on a LOAEL, which was the lowest tested dose (*i.e.,* No NOAEL is available).

Additionally, mammals are sensitive on a chronic dose basis, and larger mammals are more sensitive than small mammals. Large mammals (1000g) must consume about 0.4g bait to reach the LOAEL dose and small mammals (15g) must consume about 1.2g bait to reach the LOAEL dose (effect on decreased body weight). This is equivalent to only 1.3% of a large mammal's daily diet compared to about 40% of a small mammal's daily diet (USEPA, 1993). Therefore, based on this, risks to non-target mammals are possible, and highly likely if a mammal consumes even small amounts of bait pellets and it is much easier for larger non-target mammals to consume an effect dose (based on the LOAEL), from even only a relatively small exposure to the bait, compared to small mammals.

For potential secondary exposure to birds and mammals, considering both components of nicarbazin (DNC and HDP), which have different fate properties and likely different metabolism pathways in the birds, are needed for a successful activation of nicarbazin's MOA, reduces the likelihood of secondary exposure impact if either component is present alone. Therefore, while a predator can attack and consume a treated bird or a scavenger eating a dead treated bird (or other non-target organism) that it encounters, the differences may be enough to not make it a primary exposure route of concern.

Overall, based on the available data, if a non-target birds or mammals were to consume the nicarbazin bait, there is potential risk to birds and mammals.

The formation of the vitelline membrane is common in animals, and the mode of action of nicarbazin is to reduce the viability and hatchability of eggs by interfering with the formation of the vitelline membrane. Therefore, it is assumed that there could be adverse reproductive effects in exposed egg-laying animals other than birds, including aquatic animals both fish (surrogates for aquatic-phase amphibians) and aquatic invertebrates (water-column or sediment-phase) if exposed to nicarbazin. However, no adequate studies that evaluated reproductive effects of nicarbazin in species other than birds and mammals have been submitted or identified in the open literature. Exposure to aquatic environments may occur if nicarbazin is used in close proximity of water, or through feces of target animals because nicarbazin appears to pass through birds as DNC. Due to the fate properties of the chemical and the potential for DNC to bind to sediment, greater exposures in sediments and the subsequent risks to benthic organisms cannot be assessed due to lack of sediment toxicity data. This is a potential exposure route, but with potential exposure levels anticipated to be low. To illustrate,

a worst-case scenario is described in **Section 8** to show that it would require a rare alignment of conditions to result in appreciable exposure and as such it would be difficult to have any confidence in any aquatic exposure that was modeled or frequency of occurrence of any such modeled exposure. Therefore, EFED characterized sediment exposures as being possible but unlikely.

1.3 Environmental Fate and Exposure Summary

The nicarbazin complex breaks down into its DNC and HDP components in the digestive track. Unaltered DNC can be detected in an organism's feces. DNC, also known as N,N'-bis(4-nitrophenyl)urea or BNPU, has low solubility in water and a log octanol-water partition coefficient (log Kow) of 3.6 which suggests that DNC could bioaccumulate. HDP, also known as 4,6-dimethyl-2(1 H)-pyrimidinone, has a log Kow of -0.94 and a water solubility that is 1,000,000 times greater than DNC's, indicating that HDP is highly hydrophilic. Neither HDP nor DNC degrade via abiotic hydrolysis, while both degrade in aerobic soil metabolism with half-lives ranging from 4 to 10 days for HDP, and 200 to 2,000 days for DNC. In two submitted non-guideline terrestrial field dissipation studies, DNC dissipated with half-lives of approximately 300 days, which falls within the half-life range of DNC's laboratory aerobic soil metabolism studies.

1.4 Ecological Effects Summary

For the available aquatic studies DNC and HDP were tested separately, as DNC and HDP are not expected to not be in the water together as a complex, based on differences in aqueous solubility of the individual compounds. Therefore, the aquatic exposures of both compounds together are likely limited. HDP is practically non-toxic to freshwater fish and invertebrates on an acute basis. Acute toxicity tests for freshwater fish (warm water and cold water species) and daphnids using DNC were conducted at or above the limit of solubility. No mortality or sublethal effects reported for the freshwater fish studies. For daphnids, mortality/immobilization and sublethal effects (lethargy) were both observed in multiple test concentrations in the DNC study, whereas minimal effects were observed in the HDP study. No acute toxicity data are available for either component (DNC or HDP) for estuarine/marine fish or invertebrates; no chronic toxicity data are available for any aquatic animals, and no toxicity data is available for aquatic plants. Adverse reproductive effects in exposed egg-laying aquatic organisms are anticipated, based on the mode of action in birds. These effects cannot be quantified due to lack of chronic aquatic data.

Additionally, due to the potential of nicarbazin binding/moving into the sediment (based on the fate parameters), there is exposure potential to sediment (benthic invertebrates) as well as water column invertebrates on an acute and chronic basis. Due to the lack of sediment toxicity data, risk to sediment (benthic invertebrates) is an uncertainty. However, due to the low likelihood of exposure and limited observed acute toxicity at relatively high concentrations

(~60-90 µg/L compared to anticipated exposure), both acute and chronic risks are anticipated to be low.

For terrestrial organisms, the test material was the nicarbazin complex (DNC and HDP combined) for birds; for mammals there were a variety of studies that tested nicarbazin as a 1:1 ratio of DNC:HDP and a 3:1 ratio of DNC:HDP as well as a chronic mammalian study that tested the DNC component alone, which was the only terrestrial study available for this component of nicarbazin. Nicarbazin was practically non-toxic to mammals and birds on an acute oral basis. Nicarbazin was slightly to practically non-toxic to birds on a sub-acute dietary basis. No mortality or clinical signs of toxicity were observed in the available mammalian or avian acute oral toxicity studies. Some clinical signs of toxicity (mortality, and sublethal effects) were observed in both avian dietary studies, with greater effects observed in the mallard study.

The two-generation mammalian reproduction study used a 3:1 ratio of DNC:HDP and showed no (parental or reproductive) effects observed up the highest dose tested. Decreased body weight was identified in both the 90-day oral study and developmental toxicity studies in rats. In the developmental toxicity study which also used a 3:1 ratio of DNC:HDP, decreases in fetal weights were in the presence of maternal toxicity (decreased body weights of dams). Additionally, following 90 days of exposure with nicarbazin complex (1:1 ratio of DNC:HDP), effects included: decreases in body weights, erythrocytes, hemoglobin, and hematocrit; increases in blood urea nitrogen and plasma creatinine; increases in several relative organ weights; and tubular degeneration in the kidney and degeneration in the seminiferous tubule (in males), and a NOAEL could not be determined (*i.e.,* effects at all tested concentrations). No effects were observed in studies using the 3:1 mixture of DNC to HDP or using DNC alone. Therefore, based on the available mammalian toxicity data, nicarbazin complex (1:1 ratio of DNC:HDP) appears to be more chronically toxic than either DNC alone or the 3:1 DNC to HDP mixture (DP427299+, USEPA 2015c).

Although this chemical is registered as an egg-hatchability control agent, no guideline avian reproduction studies have been submitted to the Agency. Non-guideline studies and numerous open literature studies have been submitted on multiple species including chickens (*Gallus gallus*), and Japanese quail (*Coturnix japonica*), and in many cases demonstrate adverse reproductive effects in birds. In the recently submitted non-guideline avian reproduction study (MRID 50310301), a summary of reproduction effects on broiler breeder chickens (*Gallus gallus domesticus*) is discussed. The most sensitive endpoint was early embryonic death, which was adversely affected during the second week of treatment with nicarbazin at all dietary exposure levels (and was dose-responsive), resulting in a NOAEC that was below the lowest concentration tested (<2 mg/kg-diet). By week 4 (2 weeks post-exposure), there were no reported significant differences and a post-exposure NOAEC would be considered 40 mg/kg diet. As noted above, this is a non-guideline study and does not provide sufficient information on reproductive endpoints to meet guideline requirements. However, the study does provide information supporting the authors claim regarding the reversibility of the chemical's effects once the feed is removed from the diet. The differences in toxicity observed during exposure

and post-exposure provide some support to this argument, and this is discussed further in the risk characterization section.

No terrestrial plant or honey bee (terrestrial invertebrate) toxicity data is available for nicarbazin.

1.5 Identification of Data Needs

Fate and ecotoxicity data were identified in the generic data call-in (GDCI) as a result of the Problem Formulation for nicarbazin. Subsequent to the generic DCI, some of the guideline studies were submitted while data waiver requests were submitted by the registrant for other data. There are no fate data needs at this time. Based on the current use patterns and label directions, substantial exposure to aquatic environments is anticipated to be limited and unlikely. Therefore, additional toxicity data with aquatic organisms is not recommended at this time. The registrant did not submit avian chronic reproduction data or acute passerine data (the registrant Innolytics submitted a waiver request for the acute passerine (MRID 50310302; DP456570, USEPA, 2020) and submitted additional information in an attempt to satisfy the chronic avian reproduction study (MRID 50310301; DP454540, USEPA, 2020). Based on the mode of action for this chemical, available avian toxicity data, and potential greater sensitivity with passerines, there is potential risk for birds. The avian reproduction (OCSPP 850.2300) and acute oral passerine (OCSPP 850.2100) toxicity studies are still considered outstanding data gaps. While this data could better define toxic thresholds for surrogate test species under guideline conditions for nicarbazin, based on the mode of action for this chemical and the available avian toxicity data, submission of these studies are unlikely to change the risk conclusions for birds. Therefore, these studies are not recommended at this time. If the use pattern changes in the future, then the need for data may change.

Table 1-1. Summary of Risk Quotients for Taxonomic Groups from Current Uses of Nicarbazin

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species	Additional Information/Lines of Evidence
Freshwater Fish	Acute	Not calculated	NA	Aquatic exposure is low and considered unlikely to occur in a quantifiable amount/or in high enough amounts to pose a risk to aquatic organisms based on the use pattern and physical and chemical properties of nicarbazin (DNC and HDP).
	Chronic			
Estuarine/Marine Fish	Acute	Not calculated		
	Chronic			
Freshwater Invertebrates (Water-Column & Sediment Exposure)	Acute	Not calculated		
	Chronic	No data		
Estuarine/Marine Invertebrates (Water-Column & Sediment Exposure)	Acute	Not calculated		
	Chronic	No data		

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species	Additional Information/Lines of Evidence
Aquatic Plants	N/A	Not calculated		
Mammals	Acute	<0.02 - <0.05	No	Dose-based acute exposure/toxicity ratios from exposure of mammals were calculated as the ratio of nicarbazin intake (exposure) to the adjusted LD_{50} (toxicity). Conservatively used the non-definitive LD_{50} of >10,000 mg a.i./kg-bw as a definitive value for these calculations.
	Chronic	**1.1-2.5** (dose)	**Yes**	Dose-based chronic RQs from exposure of mammals were calculated as the ratio of nicarbazin intake (exposure) to the adjusted LOAEL (toxicity). Chronic risks to mammals could be even greater, since effects were observed in the lowest dose tested therefore, RQ values are underestimated.
Birds	Acute	<0.24 - <**0.77** (dose) **1.4** (dietary)	**Yes**	Dose-based acute exposure/toxicity ratios from exposure of birds were calculated as the ratio of nicarbazin intake (exposure) to the adjusted LD_{50} (toxicity). Conservatively assumed that the non-definitive LD_{50} of >2,250 mg a.i./kg-bw was a definitive value and assumes that birds are solely consuming nicarbazin bait. RQ exceeds the dose-based acute risk LOC (0.5) for small birds only, however, acute oral toxicity data were not available for passerines, so there is uncertainty in the relative sensitivity of potentially small birds to other birds. So while the analysis would suggest low acute dose-based risk, given the lack of passerine data there is still a potential for acute risk on an oral basis to small birds. Dietary-based sub-acute RQ compared nicarbazin concentration in bait versus the acute dietary based endpoint (LC_{50}).

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species	Additional Information/Lines of Evidence
	Chronic	**>2500x** (during exposure, based on LOAEC) **125x** (2-weeks post exposure)	**NA (see additional information)**	Dietary-based chronic qualitative analysis was completed comparing nicarbazin concentration in bait versus the chronic dietary based endpoint (NOAEC/LOAEC). Most sensitive endpoint was early embryonic mortality, with 43% higher effect at the LOAEC = 2 mg/kg-diet (during two-week exposure; NOAEC<2 mg/kg-diet), compared to control birds. Two weeks after exposure ended, there were no longer any effects on any endpoint up to the highest dose tested (40 mg/kg-diet), which is 125x below the concentration in nicarbazin granules. Given nicarbazin's MOA and high exposure/toxicity ratios, chronic risk to birds, including non-target species, is considered likely.
Terrestrial Invertebrates	Acute Adult Chronic Adult Acute Larval Chronic Larval	Not calculated	No data	Risk to terrestrial invertebrates was also not estimated due to the lack of toxicity data; however, the likelihood of exposure of terrestrial invertebrates to nicarbazin is expected to be low given the use pattern of nicarbazin.
Terrestrial Plants	N/A	Not calculated	No data	Risks to terrestrial plants was not estimated due to the lack of toxicity data; however, the likelihood of exposure of terrestrial plants to nicarbazin is expected to be low given the use pattern of nicarbazin.

Level of Concern (LOC) Definitions:
Terrestrial Animals: Terrestrial Vertebrates: Acute=0.5; Chronic=1.0; Terrestrial Invertebrates: Acute=0.4; Chronic=1.0; Aquatic Animals: Acute=0.5; Chronic=1.0; Plants: 1.0.
N/A: not applicable.
[1] RQs reflect exposure estimates for nicarbazin (DNC and HDP) and maximum application rates allowed on labels.

2 Introduction

This Draft Risk Assessment (DRA) examines the potential ecological risks associated with labeled uses of nicarbazin on non-listed non-target organisms. Federally listed threatened/endangered species ("listed") are not evaluated in this document. The DRA uses the best available scientific information on the use, environmental fate and transport, and ecological effects of nicarbazin. The general risk assessment methodology is described in the *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs* ("Overview Document")(USEPA, 2004a). Additionally, the process is consistent with other guidance produced by the Environmental Fate and Effects Division (EFED) as appropriate. When necessary, risks identified through standard risk assessment methods are further refined using available models and data. This risk

assessment incorporates the available exposure and effects data and most current modeling and methodologies.

3 Problem Formulation Update

The purpose of problem formulation is to provide the foundation for the environmental fate and ecological risk assessment being conducted for the labeled uses of nicarbazin. The problem formulation identifies the objectives for the risk assessment and provides a plan for analyzing the data and characterizing the risk. As part of the Registration Review (RR) process, a detailed Problem Formulation (USEPA, 2015b, DP427301) for this DRA was published to the docket (EPA-HQ-OPP-2015-0101-0001) in January 2016. The following sections summarize the key points of the Problem Formulation and discusses key differences between the analysis outlined there and the analysis conducted in this DRA.

Nicarbazin has previously been assessed in the Proposed Registrations of OvoControl G for geese in 2005 (DP312669, DP313794; USEPA, 2005a and 2005b), and OvoControl P for pigeons on 2007 (DP328803; USEPA, 2007a), as well as a Section 24c risk assessment on pigeons in Hawaii in 2007 (DP344692; USEPA, 2007c), and a new use risk assessment for non-native hybrid, domestic and Muscovy ducks completed in 2007 (DP340973, DP335890; USEPA, 2007b). In addition to risk assessments that were completed, supplemental reviews and follow up waiver requests have also been completed for nicarbazin (DP363859, DP363860, USEPA 2009b; DP372345, DP372348, DP349126, USEPA 2010a; DP396672, USEPA 2011b; D400098, USEPA 2012a; DP415695, USEPA 2014c; DP456570, USEPA, 2020). However, it should be noted that both the OvoControl G for geese and Muscovy duck registrations have subsequently been cancelled. Therefore, only the OvoControl P registration for pigeons (and other species) use remains.

As summarized in the Problem Formulation based on previous risk assessments, potential risks associated with the use of nicarbazin include risks to birds, and mammals, and unknown risks to benthic (sediment dwelling invertebrates) that was further examined. Fate and ecotoxicity data were identified in the generic data call-in (GDCI) document (in 2017) as a result of the Problem Formulation for nicarbazin. Subsequent to the GDCI, some of the data were submitted while data waiver requests were submitted by the registrants for other data. Fate data that has been received since the Problem Formulation are indicated below with MRIDs, and more specific information on these new data are described in in the following sections. Ecotoxicity and other relevant data that has been received since the Problem Formulation are indicated below with MRIDs, and more specific information on these new data are described in the following sections. In addition, there was a Low Volume/Minor Use Waiver Request (LV/MUWR) submitted for both effects and fate studies. The following are the guideline studies cited in the LV/MUWR OCSPP 850.1300, 850.1500, SS-1360, 850.4400, 850.4500, 850.4550, 850.1730, 835.2240, 835.4300, and 835.4400. Finally, a waiver request was submitted for the acute passerine study (MRID 50310302; DP456570, USEPA, 2020) and additional information was submitted in an attempt to satisfy the chronic avian reproduction study (MRID 50310301).

Fate and Exposure:

- Sediment and soil absorption/desorption for parent and degradate (OCSPP 835.1230)
  - Batch Equilibrium study for DNC (MRID 50310305);
  - Batch Equilibrium study for HDP (MRID 50310308);
- Soil column leaching for parent (OCSPP 835.1240)
- Hydrolysis of parent and degradates as a function of pH at 25C (OCSPP 835.2120)
- Direct photolysis rate of parent and degradates in water (OCSPP 835.2240)
- Aerobic soil metabolism (OCSPP 835.4100)
  - Aerobic soil metabolism study for DNC (MRID 50310306); and,
  - Aerobic soil metabolism study for HDP (MRID 50310309).
- Aerobic Aquatic metabolism for parent and degradates (OCSPP 835.4300)
- Anaerobic aquatic metabolism for parent and degradates (OCSPP 835.4400)
- Fish BCF for parent and DNC (OCSPP 850.1730)
- Environmental Chemistry Methods and Associated Independent Laboratory Validation (OCSPP 850.6100)

Ecotoxicity:

- Aquatic invertebrate acute toxicity test with mysid and TGAI (OCSPP 850.1035)
- Acute Sheepshead Minnow study for TGAI (OCSPP 850.1075)
- Chronic Freshwater Fish study with TGAI and DNC (OCSPP 850.1500)
- Chronic Freshwater Invertebrate study with TGAI and DNC (OCSPP 850.1300)
- Benthic Freshwater Invertebrate toxicity with TGAI and DNC (special studies – chronic)
- Aquatic plant toxicity using Lemna spp with parent and DNC (OCSPP 850.4400)
- Algal toxicity with parent and DNC (OCSPP 850.4500)
- Cyanobacteria toxicity with parent and DNC (OCSPP 850.4450)
- Avian acute oral toxicity test with TEP (MRID 50310302, DP456570, USEPA, 2020; OCSPP 850.2100)

Since the Problem Formulation was completed, the following ecotoxicity data have been submitted:

- Ecotoxicity Data
  - Non-guideline Avian Reproductive toxicity to Broiler Breeder Chickens (*Gallus gallus domesticus*); MRID 50310301, DP454540, USEPA, 2020. This study was submitted in order to fulfill the guideline requirement (*e.g.,* 850.2300) but was not conducted according to guideline recommendations and does not fulfill the requirement. The study was classified as Supplemental (QUAL), and although the study cannot be upgraded due to the lack of a definitive NOAEC, more confidence in the endpoints could be gained with the submission of the raw data and analytical measurements.
- Other Data referred to in this assessment
  - The Residue Depletion and Metabolic Identification of [^{14}C]-DNC in Chickens (*Gallus gallus domesticus*) Following Repeated Administrations of Nicarbazin-Containing [^{14}C]-DNC; MRID 50310304 (OCSPP 860.1300).

- Residue Depletion of Koffogran (Nicarbazin) in Broiler Chickens (*Gallus gallus domesticus*); MRID 50310310 (OCSPP 860.1480).
- Development and Validation of an Analytical Method for the Determination of Nicarbazin in Poultry Liver, Kidney, Muscle, Skin with Fat and Fat; MRID 50310311 (supporting analytical method).

These new data are described in more detail in the effect's characterization **(Section 6)**. Based on the current use patterns and label directions, substantial exposure to aquatic environments is anticipated to be limited and unlikely. Therefore, additional toxicity data with aquatic organisms is not recommended at this time. The avian reproduction (OCSPP 850.2300) and acute oral passerine (OCSPP 850.2100) toxicity studies are still considered outstanding data gaps. While this data could better define toxic thresholds for surrogate test species under guideline conditions for nicarbazin, based on the mode of action for this chemical and the available avian toxicity data, submission of these studies are unlikely to change the risk conclusions for birds. Therefore, these studies are not recommended at this time.

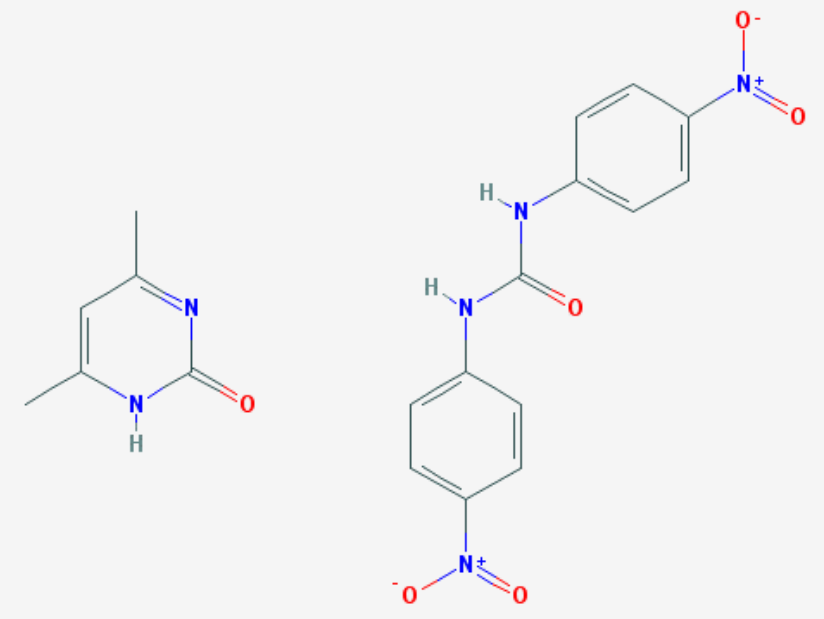
3.1 Mode of Action for Target Pests

Nicarbazin ($C_{19}H_{18}N_6O_6$, MW 426.38 g/mol) is a 1:1 complex of two compounds, 4,4'-dinitrocarbanilide (DNC, 70.89% by weight) and 4,6-dimethyl-2-pyrimidinol (HDP, 29.11% by weight). DNC is considered to be the active component, and HDP aids in its absorption. The potency of DNC is reported to be increased tenfold when it is mixed with HDP (Chapman 1994). Nicarbazin is fed to pigeons to reduce the viability and hatchability of eggs by interfering with the formation of the vitelline membrane, which separates the egg yolk and the egg white.

Historically, nicarbazin has been used since the mid-1950s as an anticoccidial agent to control intestinal protozoan parasites in the poultry industry. The Federal Drug Administration (FDA) currently approves the use of nicarbazin as a food additive for broiler chickens, but not for layers[1]. However, there is label language by FDA not to feed it to laying chickens, as it adversely affects egg production[2].

[1] https://animaldrugsatfda.fda.gov/adafda/views/#/home/previewsearch/009-476
[2] https://www.fda.gov/media/86565/download

Figure 1: Chemical structures of HDP (left) and DNC (right)[3]



3.2 Label and Use Characterization

3.2.1 Label Summary

Nicarbazin is currently registered for use as an avian contraceptive formulated as a ready-to-use control agent (treated bait) to reduce the viability and hatchability of eggs by interfering with the formation of the vitelline membrane, which separates the egg yolk and the egg white in pigeons (*Columba livia*, rock dove, feral pigeons), European starlings (*Sturnus vulgaris*), red-winged blackbirds (*Agelaius phoeniceus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), brewers blackbirds (*Euphagus cyanocephalus*), great-tailed grackles (*Quiscalus mexicanus*), boat-tailed grackles (*Quiscalus major*), common grackles (*Quiscalus quiscula*), brown-headed cowbirds (*Molothrus ater*), bronzed cowbirds (*Molothrus aeneus*), and the common myna (*Acridotheres tristis*).

Through the OvoControl P label (0.5% active ingredient; EPA Reg. No: 80224-1), nicarbazin is currently registered for use as an avian contraceptive for pigeons and other pest birds that are listed on the label. There are no agricultural pesticidal uses for nicarbazin. Applications are limited to non-food areas of manufacturing facilities, power utilities, food processing plants, hospitals, distribution centers, oil refineries, and processing centers, chemical plants, rail yards, schools, campuses, military bases, seaports, hotels, apartments, condominiums, maintenance yards, shopping malls, feed mills, airports, and other commercial, residential or industrial locations. OvoControl P may be applied via managed bait stations to rooftops, or other flat

[3] http://pubchem.ncbi.nlm.nih.gov/compound/Nicarbazin#section=Top

paved or concrete surfaces in secured areas with limited public access or areas under direct supervision of the applicator. Surfaces where birds cannot see or find the bait easily should be avoided. It is not for use in Guam, American Samoa, Northern Mariana Islands, or the U.S. Virgin Islands.

Label use directions specify:

- Applicators must ensure that children and pets do not come in contact with the bait.
- Do not apply within 20 feet of any body of water, including lakes, ponds or rivers.
- Bait must be broadcast to allow all target pest birds (*i.e.,* pigeons, starlings, blackbirds, grackles, cowbirds and mynahs) opportunity to consume the bait. A conditioning program is conducted prior to the feeding of treated bait so that the target pest birds become habituated to the daily feeding routine. Birds are fully conditioned to the baiting event when they return to the same site each day for feeding. Depending on site characteristics and target pest bird's behavior, the conditioning period can last from three (3) to thirty (30) days. If target pest birds cannot be conditioned to consume the pre-bait within 30 days, discontinue baiting at that location.
- Monitor the baiting site periodically to ensure that target pest bird and no other birds or animals are converging on the feeder or hand-broadcasted bait each morning. Do not apply if non-target feeding persists and/or cannot be prevented. Once pre-bait is consumed consistently, gradually transition the birds to OvoControl P.
- Feeding must continue DAILY through the entire breeding season, which can last all year. The daily dose (based on flock size) must be entirely consumed within 15 minutes of application. Periodically monitor the site and reconfirm target pest bird numbers. The daily amount of bait applied must be increased or decreased according to the number of target pest bird at the baiting site.
- Any accumulation of bait indicates that the birds are not consuming it regularly. In this event, remove uneaten bait and restart the conditioning program with pre-bait. Discontinue use at any location where the conditioning program fails repeatedly to consistently attract the flock.
- Depending on the geography, starlings, blackbirds, grackles, cowbirds and mynahs can breed seasonally or all year. Furthermore, subject to your location, flocks of these pest birds can be resident or migratory. OvoControl P should only be used in *resident* flocks.

- **<u>Non-Target Species:</u>**
    - Do not apply more OvoControl P than the pigeons will eat in a single feeding, as this may result in non-target species' exposure to leftover bait. Do not apply in areas where the product may be consumed by federally listed Threatened and Endangered birds.
    - Do not apply if non-target feeding persists and/or cannot be prevented. English house sparrows (*Passer domesticus*), are exempt from this restriction. *It is a violation of state and federal law to intentionally feed treated bait to non-target species, including protected species.*
- Additional requirements for use in Hawaii

- Prior to application, applicator must contact the Department of Land and Natural Resources and obtain a Wildlife Control Permit if one is required (permits are only required in Hawaii at this time).
- Do not apply in areas where Nene goose (*Nesochen sandvicensis*), Hawaiian coot (*Fulica alai*), Hawaiian moorhen (*Gallinula chloropus sandvicensis*) and Hawaiian duck (*Anas wyvilliana*) are known to occupy or graze.
- Users must notify the Pesticides Branch of the State of Hawaii Department of Agriculture, in writing prior to use. Two weeks advance notice must be given to allow time for consultation with Hawaii Department of Land and Natural Resources and the U.S. Fish and Wildlife Service.
- Observe warnings under the *Non-Target Species* section of the label. In Hawaii, Zebra doves (aka, Barred Ground dove, Blue-faced dove) *Geopelia striata*; Mountain doves (aka, Spotted dove, Chinese dove, Pearl-necked dove, Lace-necked dove) *Spilopelia chinensis*; and Common myna (aka, Common mynah, Indian myna) *Acridotheres tristis*, are exempt from the non-target restrictions.

The environmental hazard statement on the label indicates to not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean highwater mark; as well as to not contaminate water when disposing of equipment wash water or reinstate.

Table 3-1 summarizes the daily application rates provided on the label. Pursuant to the labels, once target birds are conditioned to the daily baiting program, feeding must continue daily during the entire nesting season. Depending on the climatic zone and habitat, nesting, and corresponding baiting could last year-round.

The daily application rate depends on the target species. Per the label for pigeons, the application rate is calculated using the following equation: Estimated pigeon population x 0.2 ounces (5 grams) OvoControl P = Amount of OvoControl P to be applied daily. The daily application rate for the other target birds range from 4-10 ounces of bait per 100 birds depending on the species.

Table 3-1. Summary of the Use of nicarbazin from OvoControl P labels (EPA Reg. No. 80224-1)

Target Bird	Application Rate (ounces of bait per 100 birds - unless otherwise noted)	Breeding Season
Pigeons	0.2 x entire population	Throughout the year
Starlings	8	Spring and Summer
Blackbirds	8	Spring and Summer
Grackles	10	Spring and Summer
Cowbirds	4	Spring and Summer
Mynahs	10	Throughout the year

3.2.2 Usage Summary

The Biological and Economic Analysis Division (BEAD) Chemical Profile (BCP) indicates that there is no available usage information for nicarbazin (USEPA, 2015a).

3.2.3 Label Uncertainties

Labels for nicarbazin do not limit the amount of product or active ingredient that may be applied per unit area, the number of applications that can be made per unit time, nor the minimal time interval between applications. Pursuant to the labels, once pigeons, starlings, blackbirds, grackles, cowbirds and mynahs are conditioned to the daily baiting program, feeding must continue daily during the entire nesting season.

4 Residues of Concern

In this risk assessment, the stressors are those chemicals that may exert adverse effects on non-target organisms. Collectively, the stressors of concern are known as the Residues of Concern (ROC). The ROC usually includes the active ingredient, or parent chemical, and may include one or more degradates that are observed in laboratory or field environmental fate studies. Degradates may be included in, or excluded from, the ROC based on submitted toxicity data, percent formation relative to the application rate of the parent compound, modeled exposure, and structure-activity relationships (SARs). Structure-activity analysis may be qualitative, based on retention of functional groups in the degradate, or they may be quantitative, using programs such as ECOSAR, the OECD Toolbox, ASTER, or others.

For the nicarbazin ecological risk assessment, the stressors of concern are the DNC and HDP nicarbazin components for both the aquatic and terrestrial assessments.

5 Environmental Fate Summary

Nicarbazin ($C_{19}H_{18}N_6O_6$, MW 426.38) is a 1:1 complex of two compounds, 4,4'-dinitrocarbanilide (DNC, 70.89% by weight) and 4,6-dimethyl-2-pyrimidinol (HDP, 29.1 1% by weight). It is crystalline or a yellow powder.

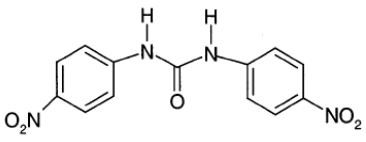
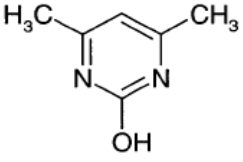
The nicarbazin complex is characterized as “inherently very stable material”, where only acidic or basic conditions led to “some” degradation, into the DNC and HDP components (MRID 46416407). The degradation of the nicarbazin complex is likely biologically mediated since DNC and HDP are detected in an organism’s feces (MRID 50310304). DNC and HDP have differing physical properties such that once in the environment, they will likely have different fate profiles.

DNC, also known as N,N'-bis(4-nitrophenyl)urea or BNPU, has the formula $C_{13}H_{10}N_4O_5$ and molecular weight of 302.25 g/mol. It has a low water solubility of 0.0465 µg/L (MRID 46416411). It has a log octanol-water partition coefficient (log Kow) of 3.6 (MRID 46416410) which suggests that DNC could bioaccumulate. Aerobic soil metabolism half-lives ranged from 200 to 2,000 days (MRID 50310306).

HDP, also known as 4,6-dimethyl-2(1 H)-pyrimidinone, has the formula $C_6H_8N_2O$, and molecular weight of 124.14 g/mol. HDP melts at approximately 200 to 205°C, has a log Kow of -0.94 (MRID 46416410), and water solubility of 19,300 µg/L (MRID 46416411). HDP's log Kow of -0.94 indicates that it is highly hydrophilic. HDP's water solubility is 1,000,000 times greater than DNC. HDP aerobic soil metabolism half-lives ranged from 5 to 10 days (MRID 50310309). Both DNC and HDP are classified as stable to abiotic hydrolysis in sterile water at pH 5 to 9 (MRID 46445305).

Relatively little is known about the environmental fate and transport characteristics of the nicarbazin complex, or its components DNC and HDP. Few OSCPP guideline compliant studies have been submitted to characterize environmental fate. Only aerobic soil metabolism and adsorption/desorption studies have been submitted since the previous risk assessments. Although additional data were called in, some of the data were waived and/or, not needed at this moment. **Table 5-1** summarizes the physical-chemical properties and environmental fate characteristics of nicarbazin and its constituents DNC and HDP. **Appendix A** provides the structures and further summary of DNC and HDP degradation products (CO_2 and unextracted residues).

Table 5 1. Physical/chemical properties and environmental fate characteristics of nicarbazin complex, DNC and HDP

Physical Property	Nicarbazin Complex	DNC Component	HDP Component	Source (MRID)
CAS Reg No.	330-95-0	587-90-6	108-79-2	--
Molecular weight, (g mol^{-1})	426.38	302.25	124.14	--
Molecular Formula	$C_{19}H_{18}N_6O_6$	$C_{13}H_{10}N_4O_5$	$C_6H_8N_{20}$	--
Structure	NA			--
Melting point (°C)	265-275	312	200-205	46416407
UV/VIS absorption Max (nm)	298	No data	300	--

Physical Property	Nicarbazin Complex	DNC Component			HDP Component			Source (MRID)
Aqueous solubility (mg/L)	No data	pH5: 2.66×10^{-5} pH7: 4.65×10^{-5} pH 9: 8.09×10^{-5}			pH5: 19.0 pH7: 19.3 pH 9: 20.0			46416411
logKow	No data	3.6			- 0.94			46416410
Vapor Pressure	No data	No data			No data			--
Henry's Law constant	No data	No data			No data			--
Hydrolysis	No data	Stable			Stable			46445305
Aerobic Soil Metabolism (days)	No data	Sandy Loam: 2230 (IORE) Sandy Clay Loam: 208 (DFOP) Silt Loam: 296 (DFOP)			Sandy Loam: 7 (IORE) Sandy Clay Loam: 10.3 (DFOP) Silt Loam: 4.41 (IORE)			DNC 50310306 HDP 50310309
Soil-Water Distribution Coefficients (Kd in L/kg-soil or sediment) Organic Carbon-Normalized Distribution Coefficients (Koc in L/kg-organic carbon)	No data	**Soil**	**Kd**	**Koc**	**Soil**	**Kd**	**Koc**	DNC 50310305 HDP 50310308
		Sandy loam	1193	91,794	Sandy loam	1.5	117	
		Clay loam	1731	55,847	Clay loam	1.1	36	
		Silt loam	1260	50,414	Silt loam	2.9	115	
		Average	1395	66,018	Average	1.83	89.3	
		Std Dev	293	22,487	Std Dev	0.945	46.2	
		CV	0.210	0.341	CV	0.516	0.517	

Abbreviations: °C = degree Celsius; g = grams; mol = mole; NA = not applicable; nm = nanometers

Two supplemental field studies were reviewed as non-guideline studies due to numerous deviations from the terrestrial field dissipation guideline. One field study (MRID 46445306) was conducted in a greenhouse using radiolabeled DNC and confined bare soil flats of silt loam (Greenfield, IN). The soil flats were confined in plastic bags to reduce volatilization and lined with aluminum foil to reduce leaching. Approximately 80 to 102% of the radioactivity applied was accounted for over a 1-year span, with a slight downward trend. The observed DT_{50} was about 28 days, however 28-30% of the applied radiation was still present as nicarbazin at 364 days, indicating that first-order kinetics were not followed.

The second field study (MRID 46416449 and 46416446) was conducted also using radiolabeled DNC in a 3-ft diameter enclosed bare plot of silt loam soil in Greenfield, Indiana. Nicarbazin fortified soil, consisting of milled, air-dried top soil (3 inches) blended with non-sterile greenhouse potting soil and chicken excreta, was incorporated at a target application rate of 2.47 kg a.i./ha in a single 3-ft diameter (7.07 sq ft) test plot surrounded by a metal culvert extending 38 cm into the soil and 23 cm above the soil. The total recovery of radioactivity from

the soil (measured as DNC) was initially 111.3% at time-0 and ranged from 112.3-142.6% throughout the remainder of the study period through 377 days post-treatment. The reviewer-calculated half-life of ^{14}C nicarbazin in soil was 301 days (r^2 = 0.80), calculated using log-linear regression analysis. At the end of the study period, 377 days post-treatment, the total carryover of residues of nicarbazin was 47.3% of the applied.

The major route of dissipation of nicarbazin under terrestrial field conditions is likely transformation and adsorption; however, samples were not analyzed for degradates. Loss from other dissipation routes (leaching, volatilization) can all be assumed to be minimal because the material balance did not decrease below the time 0 recovery of radioactivity. Dissipation due to runoff was prevented due to the design of this study (culvert around soil plot). Runoff of DNC and HDP, in either the dissolved phase or attached to soil particles or feces is a potential route of dissipation and transport to water bodies.

The available DNC terrestrial field dissipation studies with half-lives of around 300 days compare favorably with the laboratory aerobic soil metabolism half-lives for DNC that range from 200 to 2000 days.

6 Ecotoxicity Summary

Ecological effects data are used to estimate the toxicity of nicarbazin to surrogate species. The toxicity profile is not complete for nicarbazin. While acute toxicity data is available for birds, mammals, fish and aquatic invertebrates, chronic guideline toxicity data are lacking across multiple taxonomic groups. However, supplemental, non-guideline chronic toxicity data is available for birds to enable some characterization.

Ecotoxicity data for nicarbazin and its associated products have been reviewed previously in multiple ecological risk assessments with the most recent comprehensive review in 2008 (USEPA, 2008, DP Barcode 345899) and in a Problem Formulation for Registration Review (USEPA, 2015b, DP Barcode 427808). These data are summarized in **Section 6.1** and **Section 6.2.** Various studies with birds, and aquatic animals exposed to nicarbazin were received since the Problem Formulation was issued in 2015; the results of these studies are described briefly in this section and are denoted with "N" in **Table 6-1** and **Table 6-2**. The full suite of ecotoxicity data is presented in **Appendix B**.

A search of the public ECOTOXicology (ECOTOX; https://cfpub.epa.gov/ecotox/) Knowledgebase in October 2020 did not yield any useful studies with relevant toxicity information on nicarbazin. For additional information on the Endocrine Disruptor Screening Program and its potential impact on toxicity endpoints see **Appendix D**.

Table 6-1 and **Table 6-2** summarize the most sensitive measured toxicity endpoints available across taxa. These endpoints are not likely to capture the most sensitive toxicity endpoint for a particular taxon but capture the most sensitive endpoint across tested species for each taxon.

All studies in this table are classified as acceptable or supplemental. Non-definitive endpoints are designated with a greater than (>) or less than (<) value. Values that are based on newly submitted data are designated with an **N** footnote associated with the master record identification (MRID) number in tables.

6.1 Aquatic Toxicity

For the available aquatic studies DNC and HDP were tested separately. HDP is practically non-toxic to freshwater fish and invertebrates on an acute basis. Acute toxicity tests for freshwater fish (warm water and cold water species) and daphnids using DNC were conducted at or above the limit of solubility. There were no mortality or sublethal effects reported for the freshwater fish studies, however, in both the DNC and HDP bluegill studies, smaller than recommended fish were used and those studies were classified as "supplemental" rather than "acceptable." For daphnids, mortality/immobilization and sublethal effects (lethargy) were both observed in multiple test concentrations in the DNC study, minimal effects were observed in the HDP study.

No acute toxicity data are available for estuarine/marine fish or invertebrates; and no chronic toxicity data are available for any aquatic animals.

No sediment toxicity studies were submitted, and the lack of sediment toxicity data is considered an uncertainty in the aquatic toxicity dataset.

No toxicity data is available for aquatic plants.

Table 6-1. Aquatic Toxicity Endpoints Selected for Risk Estimation for Nicarbazin

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value in mg a.i./L (unless otherwise specified)	MRID or ECOTOX No./ Classification	Comments[1]
Freshwater Fish (Surrogates for Vertebrates)					
Acute	DNC (98% a.i.)	Rainbow trout (*Onchorhynchus mykiss*)	96-h LC_{50} >0.069	46416432 (Acceptable)	No mortality or sublethal effects were noted, at the reported water solubility level for DNC.
	HDP (99.4% a.i.)		96-h LC_{50} >110 (practically non-toxic)	46416431 (Acceptable)	No mortality or sublethal effects were observed.
Chronic	No data submitted				
Estuarine/Marine Fish (Surrogates for Vertebrates)					
Acute	No data submitted				
Chronic	No data submitted				

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value in mg a.i./L (unless otherwise specified)	MRID or ECOTOX No./ Classification	Comments[1]
Freshwater Invertebrates (Water-Column Exposure)					
Acute	DNC (98% a.i.)	Waterflea (*Daphnia magna*)	48-h LC_{50} >0.093	46416436 (Acceptable)	Maximum mortality was ≤25% (0.064 mg a.i./L). Lethargy was observed in the 0.064 and 0.093 mg a.i./L test concentrations. The highest concentration was above reported water solubility level for DNC.
	HDP (99.4% a.i.)		48-h LC_{50} >107 (practically non-toxic)	46416435 (Acceptable)	No treatment related mortality or sublethal effects (≤5%).
Chronic	No data submitted				
Estuarine/Marine Invertebrates (Water-Column Exposure)					
Acute	No data submitted				
Chronic	No data submitted				
Freshwater Invertebrate (Sediment Exposure)					
Chronic	No data submitted				
Estuarine/Marine Invertebrates (Sediment Exposure)					
Chronic	No data submitted				
Aquatic Plants and Algae					
Vascular	No data submitted				
Non-vascular	No data submitted				

TGAI=Technical Grade Active Ingredient; TEP= Typical end-use product; a.i.=active ingredient
>Greater than values designate non-definitive endpoints where no effects were observed at the highest level tested, or effects did not reach 50% at the highest concentration tested (USEPA, 2011a).
[1] For DNC the aqueous solubility was 2.66×10^{-5} mg/L at pH5, 4.65×10^{-5} mg/L at pH 7; and 8.09×10^{-5} mg/L at pH 9. For HDP the aqueous solubility was 19.0 mg/L at pH5; 19.3 mg/L at pH7, and 20.0 mg/L at pH 9 (MRID 46416411).

6.2 Terrestrial Toxicity

For terrestrial organisms, the test material was the nicarbazin complex (DNC and HDP combined) for birds; for mammals there were a variety of studies that tested nicarbazin as a 1:1 ratio of DNC:HDP and a 3:1 ratio of DNC:HDP as well as a chronic mammalian study that tested the DNC component alone, which was the only terrestrial study available for this component of nicarbazin. Nicarbazin was practically non-toxic to mammals on an acute oral basis. The data indicate that nicarbazin is slightly to practically non-toxic to birds on an acute dietary basis and practically non-toxic on an acute oral basis. No mortality or clinical signs of toxicity were observed in the available avian acute oral toxicity study. Some clinical signs of toxicity (mortality, and sublethal effects) were observed in both dietary studies, with greater effects observed in the mallard study as compared to the bobwhite quail study. In the mallard study, effects were first observed on Days 4 and 2, respectively, and included a slight loss of coordination, a ruffled appearance, lower limb weakness, reduced reaction to external stimuli, loss of righting reflex, lethargy, gaping, wing droop, prostrate posture, and/or thinness.

Affected birds from the 1968 ppm a.i. group generally improved during the recovery period; however, a slight loss of coordination and lower limb weakness persisted in the two surviving birds from the 5720 ppm a.i. group through test termination (LC_{50} was determined to be 3680 ppm a.i.).

Although this chemical is registered as an egg-hatchability control agent, no guideline avian reproduction studies have been submitted to the Agency. Non-guideline studies and numerous open literature studies have been submitted on multiple species including chickens (*Gallus gallus*), and Japanese quail (*Coturnix japonica*). However, the data previously submitted and reviewed do not provide sufficient information on reproductive endpoints to meet guideline requirements (see DP363859, DP363860, USEPA 2009b; DP372345, DP372348, DP349126, USEPA 2010a; DP396672, USEPA 2011b; DP400098, USEPA 2012a; and DP415695, USEPA 2014c for more detailed analysis).

The following is based on a summary from the OvoControl P for pigeons on 2007 ecological risk assessment (DP328803; USEPA, 2007a). Ott *et al.*, (1956) reported that hatchability was reduced to zero in birds fed 700 ppm nicarbazin from 1 day to 32-36 weeks of age, was decreased to about 10% of normal in birds fed 200 and 400 ppm and was impaired by about 60% at 50 or 100 ppm. The most striking and rapid effect of nicarbazin on reproduction was reduced eggshell pigmentation in hens fed 100 ppm and higher in their ration, and almost all shells were chalk white at 400 ppm. Decreased egg production and decreased egg weight was reported at 400 ppm. The potential impacts of reduced eggshell pigmentation are not known, but white eggs might be more visible and easily detected by avian, mammalian, and reptilian egg predators than would be dark or cryptically colored eggs normally laid by many bird species.

A 2006 study conducted by USDA/APHIS National Wildlife Research Center tested the effects of 5000 ppm test on pigeons (Avery 2006, MRID 46820701). Pairs of pigeons were observed during pretreatment, treatment, and recovery periods to determine the effect of 5000 ppm bait on hatchability, DNC levels in the blood and unhatched eggs, and surviving chick weight. Pairs of pigeons were fed up to 40g (8x the minimum rate on the label) of bait daily during the treatment period. DNC levels in the blood of females and daily food consumption of pairs were related to DNC levels in their unhatched eggs. No difference between mean 14-day-old chick body weights was observed between chicks hatched from the pretreatment and treatment periods. A 59% decrease in hatch was observed between the pretreatment and treatment period. No difference between mean 14-day-old chick body weights was observed between chicks hatched from the pretreatment and treatment periods. Estimated daily bait consumption ranged from 13.6 to 25.3 g per pair; thus the reduction in hatchability observed in this study was achieved with 2.7 to 5.1 times the minimum rate suggested on the proposed OvoControl P label. Further, three pairs demonstrated reduced productivity during the recovery phase, two of which laid eggs containing DNC. This result demonstrates the potential bioaccumulation of DNC and its residual effects that can occur after feeding ceases.

In a report translated from Italian and submitted by Innolytics to support the registration of nicarbazin, Martelli *et al.*, (1993) reported that nicarbazin was efficacious against pigeons in the laboratory. They fed nicarbazin to pigeons at 0, 50, 230, and 400 ppm (10 pairs per group) for the duration of the nesting period, which lasted about 120 days. According to the translation, "*even at the lowest dose used in the trials (50 ppm), the overall fertility rate (live and active nestlings/expected nestlings) is visibly reduced by up to 33.3% (average value for three nesting cycles)*." At 400 ppm, the overall fertility rate was 0%.

In contrast to the report of Martelli *et al.*, (1993), Elder (1964) reported that nicarbazin had no adverse effects on pigeon reproduction. Nicarbazin was tested as one of several possible oral contraceptives for nuisance birds. It was tested against pigeons at 100 ppm (0.01%) and 1000 ppm (0.1%) active ingredient in the diet for 19 days. At 100 ppm, all females survived and continued to lay fertile eggs at the rate of 2 clutches per month for the following 3 months. However, at 1000 ppm in the diet, 8 of the 20 pigeons died, and survivors continued to lay fertile eggs when returned to normal feed after 6 days. From the available data, there is a wide range of variability in these studies for just one tested species, pigeons.

In the recently submitted non-guideline avian reproduction study (MRID 50310301) observations included reproduction effects on broiler breeder chickens (*Gallus gallus domesticus*). Chickens were exposed to nicarbazin in daily diet for two weeks and then further observed post-exposure for an additional two-week period. The most sensitive endpoint was early embryonic death, which was adversely affected during the second week of treatment with nicarbazin at all dietary exposure levels (and was dose-responsive), resulting in a NOAEC that was below the lowest concentration tested (<2 mg/kg-diet). The percent of reported early embryonic deaths was 43, 56, 116, 128 and 334% higher for the 2, 6, 10, 25 and 40 mg/kg diet concentrations, respectively, compared to control birds, after 2 weeks of exposure. By week 4 (2 weeks post-exposure), there were no reported significant differences and a post-exposure NOAEC would be considered 40 mg/kg diet. This is considered a non-guideline study and does not provide sufficient information on reproductive endpoints to meet guideline requirements. However, the study results provide some support for the authors and registrants claims regarding the reversibility of the chemical once the feed is removed from the diet. The differences in toxicity observed during exposure and post-exposure provide some support to this argument, at least for concentrations up to 40 mg/kg, and this is discussed below in the risk characterization section.

No mortality or signs of toxicity were observed in the available mammalian acute oral toxicity study. No mortality was observed in the available mammalian acute inhalation toxicity study; there were clinical signs of toxicity that included exaggerated breathing in female rats during exposure (as of 30 minutes) and all rats starting from 2 hours into exposure. All rats exhibited no effects by day 2.

The two-generation mammalian reproduction study using a 3:1 ratio of DNC:HDP showed no (parental or reproductive) effects observed up the highest dose tested of 400 mg/kg/day (MRID 46416422). In the developmental toxicity study using a 3:1 ratio of DNC:HDP there were

decreased fetal weights observed in the presence of maternal toxicity (decreased body weights in dams; MRID 46416421). In the 90-day oral gavage study in rats with nicarbazin complex, the LOAEL was 181 mg/kg-bw/day, based on decreases in body weights, erythrocytes, hemoglobin, and hematocrit, increases in blood urea nitrogen and plasma creatinine, increases in several relative organ weights, and tubular degeneration in the kidney and degeneration in the seminiferous tubule (in males); a NOAEL could not be determined (*i.e.,* NOAEL <181 mg/kg-bw/day; EFSA, 2010; MRID 50310314). In the 90-day oral gavage study with DNC, there were no effects up to 709 mg/kg/day in rats (EFSA, 2010; MRID 50310314). Therefore, based on the available mammalian toxicity data, nicarbazin appears to be more toxic than both DNC alone and the 3:1 DNC to HDP mixture (DP427303, USEPA 2015c).

No toxicity data are available for honey bees (terrestrial invertebrates) or terrestrial plants.

Table 6-2. Terrestrial Toxicity Endpoints Selected for Risk Estimation for Nicarbazin

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Birds (Surrogates for Terrestrial Amphibians and Reptiles)					
Acute Oral	Nicarbazin complex (>99% a.i.)	Bobwhite Quail (*Colinus virginianus*)	14-days LD_{50} > 2250 mg a.i./kg-bw	46416426 (Acceptable)	Practically non-toxic; No mortality or clinical signs of toxicity were observed.
Sub-acute dietary	Nicarbazin complex (>99% a.i.)	Mallard Duck (*Anas platyrhynchos*)	8-days LC_{50} = 3680 mg a.i./kg-diet	46445302 (Acceptable)	Slightly toxic Sublethal effects included slight loss of coordination, a ruffled appearance, lower limb weakness, reduced reaction to external stimuli, loss of righting reflex, lethargy, gaping, wing droop, prostrate posture, and/or thinness. Some effects persisted through test termination.

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Chronic (reproduction)	Nicarbazin (unknown % a.i.)	Broiler breeder chicken (*Gallus gallus domesticus*)	2-week Exposure NOAEC < 2 LOAEC = 2 mg/kg-diet (early embryonic mortality (43% at the LOAEC), most sensitive endpoint) Post-exposure (2 weeks after exposure) NOAEC = 40 LOAEC >40 mg/kg-diet (no effects)	50310301[N] Supplemental (QUAL)	Other endpoints affected included fertility, and hatchability. All observed effects continued until the first week post-treatment, and by the second week post-treatment effects were comparable to control at all levels.
Mammals					
Acute Oral	Nicarbazin complex >95% a.i	Laboratory rat (*Rattus norvegicus*)	LD_{50} = >10,000 mg a.i./kg-bw (unspecified sex)	46416413 (Acceptable)	Practically non-toxic
90-day Oral	Nicarbazin Complex	Laboratory rat (*R. norvegicus*)	90-day NOAEL < 181 mg/kg-bw/d LOAEL = 181 mg/kg-bw/d	EFSA, 2010 (50310314)	Based on decreases in body weights, erythrocytes, hemoglobin, & hematocrit. Increases in blood urea nitrogen & plasma creatinine. Increases in several relative organ weights. Tubular degeneration in the kidney & degeneration in the seminiferous tubule (in males).
Terrestrial Invertebrates					
Acute contact (adult)	No data submitted				
Acute oral (adult)	No data submitted				
Chronic oral (adult)	No data submitted				
Acute oral (larval)	No data submitted				
Chronic oral (larval)	No data submitted				
Foliage Residue	No data submitted				

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Semi-field study or full field study)	No data submitted				
Terrestrial and Wetland Plants					
Seedling Emergence	No data submitted				
Vegetative Vigor	No data submitted				

TGAI=Technical Grade Active Ingredient; TEP= Typical end-use product; a.i.=active ingredient

[N] Studies submitted since the Problem Formulation was completed are designated with an N associated with the MRID number.

[1] NOAEC and LOAEC are reported in the same units.

>Greater than values designate non-definitive endpoints where no effects were observed at the highest level tested, or effects did not reach 50% at the highest concentration tested (USEPA, 2011a).

< Less than values designate non-definitive endpoints where growth, reproductive, and/or mortality effects are observed at the lowest tested concentration.

6.3 ECOSAR Analysis

An ECOSAR analysis was run for both DNC and HDP for evaluating the utility estimating toxicity for the missing aquatic toxicity data (**Appendix C**). Although the ECOSAR results would suggest low toxicity for DNC and HDP, the results appear to overestimate the solubility for both DNC and HDP. It should be noted that when compared to the empirical data, the DNC predicted data was orders of magnitude less sensitive for both fish and aquatic invertebrates; where as HDP was within the same order of magnitude of empirical data and predicted values for both fish and invertebrates. However, in light of the overestimation of the solubility for both DNC and HDP, it is not clear how reliable the results are for estimating toxicity for missing aquatic toxicity data (chronic freshwater and acute and chronic estuarine/marine data).

6.4 Incident Data

The Incident Data System (IDS) provides information on the reported ecological incidents associated with pesticides, including those that have been aggregately reported to the EPA. The database was searched in October 2020. There are no reported ecological incidents for nicarbazin in IDS.

The number of actual incidents associated with nicarbazin may be higher than what is reported to the Agency. Incidents may go unreported since side effects may not be immediately apparent or readily attributed to the use of a chemical. Although incident reporting is required under FIFRA Section 6(a)(2), the absence of reports in IDS does not indicate that the chemical has no effects on wildlife; rather, it is possible that incidents are unnoticed and unreported.

In addition to the incidents recorded in IDS, additional incidents could be reported to the Agency in aggregated form. Pesticide registrants report certain types of incidents to the Agency as aggregate counts of incidents occurring per product per quarter. Ecological incidents reported in aggregate reports include those categorized as 'minor fish and wildlife' (W-B), 'minor plant' (P-B), and 'other non-target' (ONT) incidents. 'Other non-target' incidents include reports of adverse effects to insects and other terrestrial invertebrates. However, there are no reported aggregated incidents for nicarbazin.

EPA's changes in the registrant reporting requirements for incidents in 1998 may account for a reduced number of non-aggregated reported incidents. Registrants are now only required to submit detailed information on "major" fish, wildlife, and plant incidents. Minor fish, wildlife, and plant incidents, as well as all other non-target incidents, are generally reported aggregately.

7 Analysis Plan

7.1 Overall Process

This assessment uses a weight of evidence approach that relies heavily, but not exclusively, on a risk quotient (RQ) method. RQs are calculated by dividing an estimate environmental concentration (EEC) by a toxicity endpoint (*i.*e., EEC/toxicity endpoint). This is a way to determine if an estimated concentration is expected to be above or below the concentration associated with the effects endpoint. The RQs are compared to regulatory levels of concern (LOCs). The LOCs for non-listed species are meant to be protective of community-level effects. For acute and chronic risks to vertebrates, the LOCs are 0.5 and 1.0, respectively, and for plants, the LOC is 1.0. The acute and chronic risk LOCs for bees are 0.4 and 1.0, respectively. In addition to RQs, other available data (*e.g*., incident data) can be used to help understand the potential risks associated with the use of the pesticide.

7.2 Modeling

Various models are typically used to calculate terrestrial EECs (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment). However, due to the unique use pattern for nicarbazin (treated bait fed to birds), dietary ingestion estimates were obtained from the *Wildlife Exposure Factors Handbook* (USEPA, 1993).

8 Aquatic Organisms Risk Assessment

8.1 Aquatic Exposure Assessment

Exposure to aquatic organisms was not quantitatively assessed. There are no agricultural pesticidal uses for nicarbazin. Therefore, there are two points in time when nicarbazin is

introduced into the environment and therefore, when nicarbazin might potentially be transported to waterbodies. The first would occur when the bait is broadcast for feeding. Since any uneaten bait is cleaned up at the end of the feeding period, the window of time when transport might occur is short. Since feeding occurs daily during the pigeon breeding season on an impervious surface, there is potential for rain to co-occur with feeding and transport some of the nicarbazin across the impervious surface to downstream waterbodies. However, the nicarbazin is contained in a bait matrix that would be likely to transport via runoff in only the heaviest storms. Lesser rain events may allow some of the nicarbazin to leach from the bait, but it seems unlikely that much nicarbazin transport to waterbodies would occur from the actual feeding event.

The second point in time when nicarbazin could be introduced into the environment is through the treated bird's feces. Previously nicarbazin was registered for control of geese. Because there is a definite connection between waterfowl and waterbodies, there were greater concerns for aquatic exposure from geese feces since they spend much of their time on water and defecate in water.

While pigeons and the other species listed on the label can be associated with or near aquatic habitats, they are more likely to reach nuisance levels that would require treatment only in urban or industrial terrestrial habitats. In the worst-case scenario, it could be imagined that the bait fed birds roost in marshes near the urban or industrial setting where they are causing the nuisance conditions. A small waterbody with a large treated bird population would result in the highest concentration of nicarbazin in the waterbody. Larger or flowing waterbodies would dilute any potential for a high nicarbazin concentration. While this worst-case scenario is possible, it probably would be uncommon. Therefore, it seems that most applications of nicarbazin would not pose the same risk as this worst-case scenario.

Additionally, there are reasons to believe that this worst-case scenario may pose less risk than might be imagined. First, birds do metabolize DNC before excretion. Approximately 1/3rd was metabolized, and 2/3^{rds} were excreted by chickens in a study submitted by the registrant (MRID 50310304). HDP's metabolism in birds is less well understood (due to lack of data), but given it's lower Kow, it probably is likely to be metabolized more quickly than DNC.

Second, since approximately 2/3^{rds} of the active ingredient likely passes through the pigeons unaltered via feces (MRID 50310304), the spatial extent of this secondary application of the nicarbazin in feces is uncertain, may be very non-uniform in the distribution of the feces, and likely varies greatly from one site of application to the next. Feces could be dropped over a much larger spatial scale. Assuming the birds defecate at random locations throughout their home range, it would be likely that a large fraction of the feces would fall on terrestrial environments rather than the portion occupied by the small waterbody. For some other registered species (*e.g.,* red winged blackbirds) their home range has a higher likelihood of being associated with water compared to pigeons. While some of the nicarbazin in feces that fell on a terrestrial environment may be subsequently transported to a waterbody, it is likely that much of the nicarbazin in feces that falls on the terrestrial environment would be trapped

by vegetation or soil on pervious surfaces and would contribute little to aquatic exposure. Again, it should be noted, that there are no agricultural pesticidal uses for nicarbazin. Although the label restricts the applicator from applying within 20 feet of any body of water, birds are not under this constraint for secondary application via feces. Therefore, even though the label appears to be very prescriptive, the actual environmental distribution of the active ingredient is determined by an uncontrolled flock of wild animals.

While any impervious surfaces could lead to greater transport of feces to waterbodies, a small waterbody (necessary for high aquatic concentrations) that has more impervious surfaces draining to it will frequently overflow (transport through the waterbody rather than to the waterbody) due to the substantial runoff input. Therefore, a small waterbody that accumulates HDP and DNC could not have much impervious surface drainage. A small waterbody that doesn't overflow could theoretically have 100% impervious surfaces if it had a small enough watershed draining to it. However, as the waterbody drainage area becomes smaller, it becomes less likely that the randomly dropped feces would fall on it. Any feces falling outside of the waterbody and its drainage area would not contribute to aquatic exposure.

Third, HDP is metabolized much quicker in the aerobic soil metabolism studies than DNC. If HDP promotes the toxicity of DNC in aquatic organisms (as it does for birds) but degrades at a much faster rate in the environment, or differences in mobility result in less transport of one of the components than the other, it is likely that HDP and DNC would not occur in the most toxic ratio. Therefore, in the presence of reduced HDP concentrations, a higher concentration of DNC might be required to elicit the same toxic effect.

Because of the large number of factors that would need to co-occur in order to produce a potential scenario that might pose risk and the uncertainty in any parameters that might be used to model such a scenario, it would be difficult to have any confidence in any generated aquatic exposure estimates. Therefore, EFED has characterized such exposures as possible but unlikely to occur and unlikely to result in sufficient DNC and HDP exposure in a small enough waterbody to reach toxic concentrations.

An uncertainty with this conclusion on the likelihood of aquatic exposure is that multiple bird species are listed on the label as target species. An internet search of the other target species indicates that many of these species' habitats may overlap with aquatic resources to a greater extent than pigeons. For example, red-winged blackbirds are commonly sighted on aquatic vegetation near waterbodies. None seemed as intimately connected to aquatic environments as waterfowl, which represented a greater concern when nicarbazin was registered for geese (*e.g.*, the geese were known to defecate directly into the water where they spent much of their time). Therefore, it seems reasonable to characterize EFED's confidence as "possible, but unlikely aquatic exposure" as confident for pigeons, but somewhat less confident in regard to the other target species; however, it is still considered unlikely to result in sufficient aquatic exposure concentrations.

8.1.1 Monitoring

The Water Quality Portal (USEPA *et al*., 2018.)[4] was searched for monitoring information on nicarbazin and its individual components (HDP and DNC) in November 2020. No monitoring data was found.

8.2 Aquatic Organism Risk Characterization

Risk to aquatic organisms was not quantitatively assessed given the lack of confidence in estimating aquatic exposures. Also, while exposure is possible under certain worst-case conditions described above, it is unlikely to occur or result in sufficient exposures to reach toxic concentrations. As a worst-case scenario, any residues would not result in an appreciable amount, as such it would be difficult to have any confidence in any aquatic exposure that was generated. Therefore, such exposures are characterized as being possible but unlikely. For freshwater fish (surrogates for aquatic-phase amphibians) and aquatic invertebrates, acute toxicity values were non-definitive, indicating low potential for acute risk. Aquatic organisms are not expected to be an acute risk as aquatic exposure is assumed to be limited. However, due to the lack of chronic toxicity data, chronic risk to aquatic organisms is an uncertainty. Additionally, there are no aquatic plant toxicity data. However, due to the low likelihood of exposure, consequently both acute and chronic risks to aquatic organisms are anticipated to be low. As a worst-case scenario, any residues would not result in an appreciable amount, as such it would be difficult to have any confidence in any aquatic exposure that was generated. Therefore, such exposures are characterized as being possible but unlikely.

9 Terrestrial Vertebrates Risk Assessment

This assessment of the labeled uses of nicarbazin relies on the deterministic RQ method to provide a metric of potential risks. The RQ provides a comparison of exposure estimates to toxicity endpoints (*i.e.*, estimated exposures divided by acute and/or chronic toxicity endpoints expressed in the same units as exposures, respectively). The resulting unitless RQ values are in turn compared to the Agency's LOCs. EPA uses the LOCs to indicate when the use of a pesticide, as directed by the label, has the potential to result in exposure levels sufficient to cause adverse effects to non-target organisms. A discussion of the RQ values and a qualitative analysis for nicarbazin and other information that provides context for the interpretation of potential risk to various taxa are presented below.

Nicarbazin risk to birds and mammals was evaluated under the assumption that birds and mammals can access nicarbazin bait despite the requirement/recommendation that bait be applied in manners to reduce exposure (*i.e.,* clean-up of unused bait, discontinuation in the presence of non-target species, etc.).

[4] https://www.waterqualitydata.us/

9.1 Terrestrial Vertebrate Exposure Assessment

Primary exposure is assessed in this risk assessment and is defined as consumption of treated bait/pellets by target or non-target organisms. Secondary exposure in this assessment is defined as predation and consumption of exposed primary consumers. Exposure (food dry weight consumption) estimates were derived using allometric equations from USEPA's *Wildlife Exposure Factor Handbook* (USEPA, 1993).

Primary exposure through nicarbazin bait consumption was calculated using two methodologies. For the first method, nicarbazin exposure was calculated as mg a.i./kg-bw/day, where kg-bw is the weight of the consuming individual for three standard weight classes of birds and mammals. Exposure (food dry weight consumption) estimates were derived using allometric equations from USEPA's *Wildlife Exposure Factor Handbook* (USEPA, 1993). The allometric equations for birds and mammals were used to approximate those individuals with a high potential for consuming food and thus give the most conservative exposure estimates. Formulas for calculation of intake estimates are provided in **Table 9-1**, and nicarbazin exposure estimates (on a dose basis) are provided in **Table 9-2**.

For the second method of exposure calculation, the amount of bait that a bird and/or mammal (*e.g.,* rodent) would have to consume to reach the LD_{50} can be calculated; this information was used to understand the amount of bait that a non-target bird and/or mammal would need to consume to reach a lethal dose. This dietary exposure value can then be compared to the daily food intake for different sizes of birds (20-1000 g) or mammals (15-1000 g). For both birds and mammals, the amount of bait that a bird or mammal would have to consume to reach the non-definitive LD_{50} (greater than 2250 mg a.i./kg-bw; or 10,000 mg a.i./kg-bw, respectively) value can be estimated conservatively, since the acute toxicity values are non-definitive for both birds and mammals, this can be considered a conservative estimate of acute risk. For birds, passeriform birds were used as a surrogate bird taxon in the dietary intake formulas below to represent the most common (and sensitive) non-target birds likely to feed on nicarbazin bait. Likewise for mammals, rodents were used as the surrogate mammalian taxa in the dietary intake formulas below to represent the most common (and sensitive) non-target mammals likely to feed on nicarbazin bait.

The EEC for direct effects to birds and mammals was calculated based on EFED's default body weight classes for birds and mammals. For birds the weight classes are small (20 g), medium (100 g), and large (1000 g), and for mammals the weight classes are small (15 g), medium (35 g), and large (1000 g).

Table 9-1. Formulas for Calculating Nicarbazin Intake for Birds and Mammals Based on Consumption of Bait

Passeriform bird food intake (g, dry weight): FI (g dry-wt/day) = 0.398 * $Wt(g)^{0.850}$
Mammal (rodent) food intake (g, dry weight): FI (g dry-wt/day) = 0.621 * $Wt(g)^{0.564}$
Nicarbazin intake (mg a.i./kg-bwt/day) = FI (g dry-wt/day) * mg a.i./kg-bait / Wt(g)
Where: Wt (g) = weight (in grams) of the bird or mammal consumer

Food intake equation is from the *Wildlife Exposure Factors Handbook* (USEPA, 1993).

Table 9-2. Expected Nicarbazin Intake for Birds and Mammals Based on Primary Consumption of Bait

Species or Taxa	% a.i. in bait	Nicarbazin Concentration in Bait (mg a.i./kg-bait)[1]	Body Weight (g)	Daily Food Intake (g/day)*	Nicarbazin Intake (mg a.i./kg-bw/day)**
Birds	0.5	5,000	20	5	1,250
			100	20	1,000
			1000	141	705
Mammals			15	3	1,000
			35	5	714
			1000	31	155

*See **Table 9-1** for derivation.

** (Concentration in bait) * (daily food intake) / (Wt(g)).

1 kg of bait containing 0.5% ai contains 5,000 mg of nicarbazin.

Table 9-3. Formulas for Calculation of Weight-Adjusted Mammalian Nicarbazin Lethal Doses for 50% of the Animals Tested (LD_{50})

Adjusted Mammalian LD_{50}

$$\text{Adj. } LD_{50} = LD_{50}\,(TW/AW)^{(0.25)}$$

Where:

Adj. LD_{50} = adjusted LD_{50} (mg a.i./kg-bw)

LD_{50} = endpoint reported from mammal study (10,000 mg a.i./kg-bw)[1]

TW = body weight of tested animal (350 g rat Wistar)[2]

AW = body weight of assessed animal (g)

From: *Wildlife Exposure Factors Handbook* (USEPA, 1993).

[1] Conservatively uses the highest dose tested (10,000 mg a.i./kg-bw; MRID 46416413), which resulted in no mortality, as the endpoint to represent potential acute risks.

Table 9-4. Formulas for Calculation of Weight-Adjusted Avian Nicarbazin LD_{50}'s

Adjusted Avian LD_{50}

$$\text{Adj. } LD_{50} = LD_{50}\,(AW/TW)^{(0.15)}$$

Where:

Adj. LD_{50} = adjusted LD_{50} (mg a.i./kg-bw)

LD_{50} = endpoint reported from avian study (2,250 mg a.i./kg-bw)[1]

TW = body weight of tested animal (178 g Northern bobwhite)

AW = body weight of assessed animal (g)

From: Mineau *et al* (1996).

[1] Conservatively uses the highest dose tested (2,250 mg a.i./kg-bw; MRID 46416426), which resulted in no mortality, as the endpoint to represent potential acute risks.

Table 9-5. Formula for Calculation of Amount of Bait Consumed to Reach LD_{50} Dose for Mammals

Amount of Bait to be Consumed to Reach LD_{50} Dose (g bait/animal)

$$= \text{Adj. } LD_{50} \times AW / \text{Concentration in Bait}$$

Where:

Adj. LD_{50} = adjusted LD_{50} (mg a.i./kg-bw)[1]

LD_{50} = endpoint reported from mammal study (>10,000 mg a.i./kg-bw)

AW = body weight of assessed animal (kg)

Concentration a.i. in bait (mg a.i./g bait)

[1]. See **Table 9-7** for adjusted LD_{50} values.

Table 9-6. Formula for Calculation of Amount of Bait Consumed to Reach LD_{50} Dose for Birds

Amount of Bait to be Consumed to Reach LD_{50} Dose (g bait/animal) =Adj. LD_{50} x AW / Concentration in Bait *Where:* Adj. LD_{50} = adjusted LD50 (mg a.i./kg-bw)[1] LD_{50} = endpoint reported from avian study (2250 mg a.i./kg-bw) AW = body weight of assessed animal (kg) Concentration a.i. in bait (mg a.i./g bait)

[1]. See **Table 9-7** for adjusted LD_{50} values.

9.2 Terrestrial Vertebrate Risk Characterization

As described above, two methods are used to assess risks to birds and mammals from the use of nicarbazin as an avian contraceptive. Since the bait is applied in bait stations as a means to try and prevent exposure, drift will not occur, and runoff are not expected except under worst case scenarios and are not considered exposure pathways.

Dose-based acute exposure/toxicity ratios from exposure of birds and mammals were calculated as the ratio of nicarbazin intake (exposure) to the adjusted LD_{50} (toxicity). The risk analysis assumed that non-target birds and mammals are able to access nicarbazin bait at the site of application, by accessing bait pellets placed on the ground/in bait pans, assuming 100% of diet is bait, this would represent a worst case scenario condition.

For the acute risk analysis for birds, it was assumed that the non-definitive LD_{50} of >2,250 mg a.i./kg-bw was a definitive value (*i.e.,* LD_{50}=2,250 mg a.i./kg-bw) in order to calculate acute an exposure/toxicity ratio for birds. This approach results in a highly conservative estimate of acute risk to birds, representing a worst case scenario condition based on the information available. For the acute risk analysis for mammals, it was similarly assumed that the non-definitive LD_{50} of >10,000 mg a.i./kg-bw was a definitive value (*i.e.,* LD_{50}=10,000 mg a.i./kg-bw) in order to calculate an acute exposure/toxicity ratio for mammals. This approach results in a highly conservative estimate of acute risk to mammals.

The acute exposure/toxicity ratios for birds and mammals would be above the relevant acute risk LOC (0.5) for small birds only (the exposure/toxicity ratios range from <0.02 – <**0.77**; **Table 9-7**). Again this approach uses non-definitive LD_{50} values as definitive values and results in conservative estimates of acute risk to both birds and mammals, based on the information available. However, it is noted that acute oral passerine data was not submitted, therefore there is some uncertainty as to whether passerine species may be more sensitive then what the data for quail and duck indicate, and therefore risk to passerine (and potentially other small birds) cannot be precluded on an acute oral basis from the exposure/toxicity ratios alone.

Dose-based chronic RQs from exposure of mammals were calculated as the ratio of nicarbazin intake (exposure) to the adjusted LOAEL (toxicity). The risk analysis assumed that non-target

mammals are able to access nicarbazin bait at the site of application, the bait by accessing bait pellets placed on the ground/in bait pans.

For the chronic risk analysis for mammals, the lowest mammalian endpoint, LOAEL of 181 mg a.i./kg/day was used to in order to calculate chronic dose-based RQs for mammals (as a NOAEL could not be determined). This approach results in a potentially less conservative estimate of chronic risk to mammals. The chronic risk estimates for mammals exceed the chronic risk LOC (1.0) for all sizes of mammals assessed, RQ values (range: **1.1** – **2.5**) (**Table 9-7**). Based on these results, actual chronic risk to mammals could be even greater, as the exposure/toxicity ratios presented here underestimate risk compared to no effect thresholds (which the Agency uses to estimate risk) since chronic effects on body weight were observed at all doses in the available dataset. This endpoint is also based on the 90-day developmental study as a 2-generation study with nicarbazin complex is not available, which adds to the uncertainty surrounding the exposure to toxicity estimates of risk.

Table 9-7. Exposure to Toxicity Ratios for Birds and Mammals Based on Consumption of Bait

Species or Taxa	Nicarbazin Concentration in Bait (mg a.i./kg-bait)	Weight (g)	Nicarbazin Intake (mg a.i./kg-bw/day)[1]	Adjusted LD_{50} (mg a.i./ kg-bw)[2]	Adjusted LOAEL (mg a.i./kg-bw)[4]	Dose-based Acute Exposure/ Toxicity Ratios [3]	Dose-based Chronic RQ[4]
Birds	5,000	20	1,250	>1621	N/A	<**0.77**	N/A
		100	1,000	>2064	N/A	<0.48	N/A
		1000	705	>2915	N/A	<0.24	N/A
Mammals		15	1,000	>21978	398	<0.05	**2.5**
		35	714	>17783	322	<0.04	**2.2**
		1000	155	>7692	139	<0.02	**1.1**

1 See **Table 9-1** for derivation.
2 See **Table 9-3** and **Table 9-4** for derivation. Used the body weight of the test animal for calculations of adjusted dose.
3 RQ= Nicarbazin Intake/Adjusted LD_{50}. **Bolded exposure/toxicity ratios** exceed the acute risk to non-listed species level of concern (LOC) of 0.5.
4 RQ = Nicarbazin Intake/Adjusted LOAEL. **Bolded RQs** exceed the chronic risk LOC (1.0); the most sensitive dose-based endpoint for mammals was the 90-day oral gavage study with the nicarbazin complex, the LOAEL 181 mg/kg/day; a NOAEL could not be determined; <181 mg/kg-bw/day (EFSA, 2010; MRID 50310314).

As described earlier, a second method to evaluate primary exposure was a dietary approach using the amount of nicarbazin bait needed to be consumed to reach the LD_{50} dose which can then be compared to the daily food intake.

The amount of bait that a mammal would have to consume to reach the LD_{50} of >10,000 mg a.i./kg and >2,250 mg/kg-bw for birds was calculated. Exposure (food dry weight consumption) estimates were derived using allometric equations from *The Wildlife Exposure Factors Handbook* (USEPA, 1993). The allometric equations for passeriform birds and small mammals (rodents) were used as these would best approximate those individuals with high potential for consuming grain, and they would give the most conservative exposure estimates. Food dry

weight was assumed equivalent to food wet weights as the expected water content of the bait would be minimal.

For both birds and mammals, no definitive dose-based acute toxicity data are available. However, if it is assumed that the LD_{50} is 2,250 mg/kg-bw for birds and the LD_{50} is 10,000 mg/kg-bw for mammals (the highest tested doses in the acute toxicity studies), the amount of bait that a bird and/or mammal would have to consume to reach that dose level can be calculated. These estimations would be conservative calculations, representing worst case scenarios given that the no mortalities were observed at these doses. Formulae for calculation of dose estimates are provided in **Table 9-5** and **Table 9-6** above. **Table 9-7** provides a summary of these estimates. This analysis indicates that compared to the daily food intake for various sized birds and mammals, a varying amount of bait (*e.g.,* 6.5 g for small birds, and 65.9 g for small mammals) is needed to reach the non-definitive LD_{50} values.

As can be seen in **Table 9-8**, for birds, the amount of bait to be consumed to reach the LD_{50} is larger than the daily food intake, ranging from 129.7% for small birds to about 413.5% of the daily food intake for larger birds, indicating that it is unlikely for birds to consume a lethal dose (based on the non-definitive endpoint where no mortality occurred). However, since passerine acute oral toxicity data were not available, there is uncertainty in whether a smaller proportion of diet may be necessary to reach passerine toxicity endpoints, compared to the tested duck and mallard species. The data suggest that it would be unrealistic for non-passerine species to consume sufficient diet from their daily intake to reach a lethal dose, but it is considered an uncertainty for smaller passerine species alone.

For mammals, the amount of bait to be consumed to reach the LD_{50} is much larger than the daily food intake, ranging from 2197.8% for small mammals to about 4962.3% of the daily food intake for larger for mammals, indicating that, as for non-target birds, it is unlikely for non-target mammals to consume a lethal dose. However, for mammals, the amount of bait to be consumed to reach the chronic LOAEL is much less than the daily food intake, ranging from only 1.3% for large mammals to about 39.8% of the daily food intake for smaller mammals, indicating that it is much easier for larger non-target mammals to consume a lethal dose.

Table 9-8. Amount of Bait to be Consumed to Reach LD_{50} Dose for Birds and Mammals and LOAEL for Mammals

Species or Taxa	Nicarbazin Concentration in Bait (mg a.i./ g-bait)	Weight (g)	Adjusted LD_{50} (mg a.i./ kg-bw)[1]	Adjusted LOAEL (mg a.i./ kg-bw)[1]	Amount of Bait to be Consumed to Reach Adjusted LD_{50} (g bait)[2]	Amount of Bait to be Consumed to Reach Adjusted LOAEL (g bait)	Daily food Intake (g)[3]	Percentage of Daily Food Intake (to reach LD_{50})	Percentage of Daily Food Intake (to reach LOAEL)
Birds	5000	20	>1621	N/A	6.5	N/A	5.1	129.7%	N/A
		100	>2064	N/A	41.3	N/A	20	206.4%	N/A
		1000	>2915	N/A	583.0	N/A	141	413.5%	N/A
Mammals		15	>21978	398	65.9	1.2	3	2197.8%	39.8%
		35	>17783	322	124.5	1.0	5	2489.6%	19.3%
		1000	>7692	139	1538.3	0.4	31	4962.3%	1.3%

[1] See **Table 9-7** for adjusted LD_{50} and LOEAL values.
[2] See **Table 9-5** and **Table 9-6** for derivation.
[3] See **Table 9-1** for derivation.

In addition to the analysis completed above using the acute oral toxicity study, to estimate risks to birds from both acute and chronic exposure an analysis was completed comparing nicarbazin concentration in bait versus the acute or chronic dietary based endpoint (LC_{50} or NOAEL/LOAEL).

A comparison of the 5,000 mg/kg-diet in bait versus 3680 mg a.i./kg-diet in duck results in a sub-acute dietary RQ of **1.4**. This indicates that there is potential for sub-acute dietary risk resulting from dietary exposure to non-target birds consuming treated bait.

There are no acceptable guideline studies evaluating avian reproduction available. Therefore, in the absence of studies that fulfill guideline requirements, the recently submitted and reviewed chicken reproduction study (MRID 50310301), which provides supplemental reproductive endpoints, was used to evaluate the chronic risk to birds on a qualitative basis.

This study is only a 2-week exposure period, versus the typical 20-week exposure period found in guideline avian reproduction studies. In addition, the test species that was used, broiler breeder chickens (*Gallus gallus domesticus*), as compared to typical avian test species (*e.g.,* northern bobwhite quail and/or mallard ducks). Chickens are not a typical test species for evaluating chronic effects of pesticides to birds and there is additional uncertainty in extrapolating effects from this species across the taxa compared to the standard surrogate test species for which data are typically generated to evaluate avian toxicity. The measured endpoints that were reported in the study included fertility, hatchability (% hatch of fertile eggs and % hatch of eggs set), cracked eggs, eggshell pigmentation, and early embryonic mortality. Other endpoints that were not included or measured in this study that are recommended by the OCSPP 850.2300 guideline and typically included in an acceptable guideline avian reproduction study; include the following: growth, eggshell thickness, hatchling survival, eggs laid/pen, body weight (initial, and/or hatchlings), embryo measurements, etc. Without the inclusion of the additional recommended endpoints a full risk picture of avian reproduction cannot be fully understood. One benefit however, of this study is that although this study only had a 2-week exposure period, during which time the test species were fed nicarbazin daily for the entire exposure period and measured reproductive endpoints were recorded and reported (NOAEC <2 mg/kg-diet, LOAEC = 2 mg/kg-diet) after the 2 week exposure, the test species were fed a clean diet, and a 2-week post-exposure NOAEC was also reported (40 mg/kg-diet). This post-exposure measurement provides additional information, highlighting the differences between during- and post- exposure effects. Overall, this study found that when the nicarbazin bait is removed from the diet, two-weeks after exposure, treatment related effects were not observed on any measured endpoint.

The results of the chicken study can only be used qualitatively to compare the nicarbazin concentration in bait (5,000 mg/kg-diet in bait) versus the chronic endpoint (during exposure

NOAEC <2 mg/kg-diet). To estimate the chronic dietary-based risk, the resulting concentration in bait was 2500x the chicken reproductive endpoint, since the NOAEC is a non-definitive endpoint (effects were observed in all treatment levels, and was dose-responsive, with the most sensitive endpoint being early embryonic mortality, with 43% effects at the LOAEC = 2 mg/kg-diet). For additional characterization, the chronic dietary-based risk was also estimated qualitatively for the 2 weeks post-exposure period using the nicarbazin concentration in bait (5,000 mg/kg-diet in bait) versus the chronic endpoint (2 weeks post-exposure NOAEC = 40 mg/kg-diet, the highest dose tested). The resulting concentration in bait was 125x the chicken reproductive endpoint, (where no effects were observed in any treatment levels 2 weeks after exposure had ended).

Data are available demonstrating that non-target species of birds and mammals consumed nicarbazin bait in a field study designed to test efficacy in Canada geese (not a currently registered use; Bynum *et al.*, 2005 [MRID 46497103]). Although the application conditions in the field study were different than actual label-compliant use for the control of pigeons, it can be concluded that direct exposure to the nicarbazin complex by non-target species through the consumption of treated bait is not precluded by any characteristics of the bait itself (*i.e.*, the bait can be consumed by a variety of organisms).

The degree to which non-target species may be directly exposed to nicarbazin depends upon, but is not limited to, the applicator's compliance with label use directions and prohibitions, the presence of mixed flocks, the amount of treated bait consumed, and if the unconsumed bait is adequately monitored and removed from the treatment site.

Therefore, based on the available data, while exposures may be limited if used in accordance to the label, if birds and mammals did consume the nicarbazin bait, there may be potential risk to birds and mammals.

Secondary Toxicity

Secondary exposure is also possible (EFSA 2003), but the extent to which this may occur is unknown. Nicarbazin and DNC released into the terrestrial environment from bait or feces will be available to aquatic organisms and soil macroinvertebrates for uptake or consumption. Terrestrial animals that feed on soil macroinvertebrates may receive exposure via this pathway. Secondary exposure may also occur to predators and scavengers consuming tissues and/or eggs of animals that have ingested bait. This exposure may occur in both the aquatic and terrestrial environment and may raise the potential for risk to species that consume eggs of target and non-target birds (*e.g.*, birds and reptiles). As discussed above, biomagnification and food-chain exposure of predators is possible if exposure is continuous.

Both components of nicarbazin (DNC and HDP), which have different fate properties and likely different metabolism pathways in the birds, are needed for a successful activation of nicarbazin's MOA, which reduces the likelihood of secondary exposure impact if either component is present alone. So, while a predator can attack and consume a treated bird or a

scavenger eating a dead treated bird (or other non-target organism) it comes across, the differences may be enough to not make it a primary exposure route of concern.

Based on the chicken metabolism study (MRID 50310304) results, the metabolic pathway of the DNC component of nicarbazin in poultry involves acetylation of one of the nitrogen dioxide groups to form the acetyl derivative of DNC. DNC and its acetyl derivative were the primary components identified in excreta, accounting for 64.4% and 1.7% total radioactive residues (TRR), respectively, in excreta collected from Group 3 males one day following the initial dose. By withdrawal day 4, residues in all tissues (liver, kidney, skin, fat, and excreta) declined to 0.02-0.03x the levels observed at withdrawal day 0, and by withdrawal day 9, residues had declined to 0.001-0.005x the original levels (MRID 50310304). This study suggests that based on the DNC component, and the metabolism within poultry, there is reduced likelihood of secondary exposure impact, at least if the DNC component is present alone.

9.3 Terrestrial Plant and Invertebrate Risk Characterization

Risks to terrestrial plants were not estimated due to the lack of toxicity data; however, the likelihood of exposure of terrestrial plants to nicarbazin is expected to be low given the use pattern of nicarbazin. Similarly, risk to terrestrial invertebrates was also not estimated due to the lack of toxicity data; however, the likelihood of exposure of terrestrial invertebrates to nicarbazin is expected to be low given the use pattern of nicarbazin.

10 Conclusions

Consistent with previous risk assessments (USEPA, 2015b), there is a potential for direct adverse effects to birds and mammals from exposure to nicarbazin as a result of registered uses. However, in general, when used in accordance with the label, although some of these statements may be viewed as more advisory rather than mandatory (in secured areas with limited public access or areas under direct supervision of the applicator, with excess bait removed and feeding halted if non-target birds and mammals present at time of application), it is likely that non-target wildlife exposures will be limited.

Risks to terrestrial plants and terrestrial invertebrates, including pollinators, are not expected to be at risk due to a lack of significant exposure, given the use pattern of nicarbazin. Aquatic organisms are not expected to be an acute risk as aquatic exposure is assumed to be negligible. However, due to the lack of chronic toxicity data, chronic risk to aquatic organisms is an uncertainty (additionally, there are no aquatic plant toxicity data). As a worst-case scenario, any residues would not result in an appreciable amount, as such it would be difficult to have any confidence in any aquatic exposure that was generated. Therefore, such exposures are characterized as being possible but unlikely, as such, due to the low likelihood of exposure, consequently both acute and chronic risks are anticipated to be low.

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MRID **Citation Reference**

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MRID	Citation Reference
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46416422	Messina, J. (2004) Nicarbazin Multigeneration Reproduction Toxicology. Project Number: NICARBAZIN/04/05. Unpublished study prepared by Exponent. 48 p.
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MRID	Citation Reference
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50310311	MacDonald, A. (2017) Development and Validation of an Analytical Method for the Determination of Nicarbazin in Poultry Liver, Kidney, Muscle, Skin with Fat and Fat. Project Number: 25477, 207193. Unpublished study prepared by Inveresk Research International. 87p.
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50310314	EFSA (2010) Scientific Opinion on the Safety and Efficacy of Koffogran (Nicarbazin) as a Feed Additive for Chickens for Fattening.
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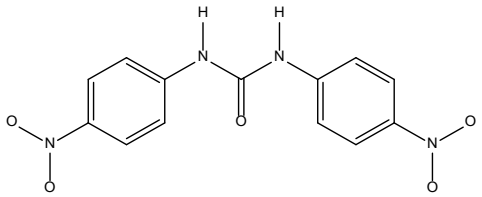
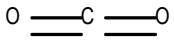
50931201	Greenslees, K. (2018) Finding of No Significant Impact (FONSI) for Maxiban (Narasin and Nicarbazin) in Chickens. Project Number: NICARABAZIN. Unpublished study prepared by Lilly Research Laboratories. 7p.
50931202	Perkins, A. (2019) Environmental Assessment of the Use of Maxiban (Narasin and Nicarbazin) in Feed for Prevention of Coccidiosis in Broiler Chickens. Project Number: NICARBAZIN. Unpublished study prepared by Lilly Research Laboratories. 160p.
50931203	US Food and Drug Administration (2018) Freedom of Information Summary ("FOI") Maxiban (Narasin and Nicarbazin) NADA 138-952. Project Number: NICARBAZIN, NADA/138/952. Unpublished study prepared by U.S. Food & Drug Administration. 46p.

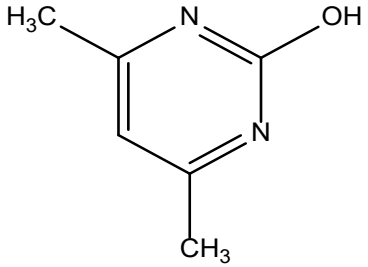
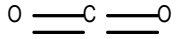
Appendix A. ROCKS table

Table A1. Chemical Names and Structures of Component DNC and its Transformation Products

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)		Final %AR (study length)
PARENT							
1,3-Bis-(4-nitrophenyl) urea (4,4'-Dinitrocarbanilide; DNC)	IUPAC: 1,3-Bis(4-nitrophenyl)urea **CAS No.:** 587-90-6 **Formula:** $C_{13}H_{10}N_4O_5$ **MW:** 302.25 g/mol **SMILES:** [H]N(C(N(C1=CC=C(N([O])[O])C=C1)[H])=O)C2=CC=C(N([O])[O])C=C2		835.2120 Hydrolysis	46445305	pH 5	PRT	106.3% (30 d)
					pH 7	PRT	115.4% (30 d)
					pH 9	PRT	99.6% (30 d)
			835.4100 Aerobic soil metabolism	50310306	Sandy loam	PRT	72.23% (120 d)
					Sandy clay loam	PRT	61.57% (120 d)
					Silt loam	PRT	99.6% (120 d)
MAJOR (>10%) TRANSFORMATION PRODUCTS							
Unextractable residues	NA	NA	835.4100 Aerobic soil metabolism	50310306	Sandy loam	**27.30%** (120 d)	**27.30%** (120 d)
MINOR (<10%) TRANSFORMATION PRODUCTS							
Carbon dioxide	Carbon dioxide **Formula:** CO_2 **MW:** 44 g/mol **SMILES:** C(=O)=O		835.4100 Aerobic soil metabolism	50310306	Sandy loam	1.12% (120 d)	1.12% (120 d)
					Sandy clay loam	0.81% (120 d)	0.81% (120 d)
					Silt loam	1.96% (120 d)	1.96% (120 d)

ND= means "not detected". AR means "applied radioactivity". MW means "molecular weight". LOQ means "limit of quantitation". Bolded values are laboratory study values >10%AR.

Table A2. Chemical Names and Structures of Component HDP and its Transformation Products

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)		Final %AR (study length)
PARENT							
2-Hydroxy-4,6-dimethyl pyrimidine (HDP)	**IUPAC:** 4,6-Dimethylpyrimidin-2-ol **CAS No.:** 108-79-2 **Formula:** $C_6H_8N_2O$ **MW:** 124.14 g/mol **SMILES:** OC1=NC(C)=CC(C)=N1		835.2120 Hydrolysis	46445305	pH 5	PRT	98.7% (30 d)
					pH 7	PRT	100.2% (30 d)
					pH 9	PRT	111.8% (30 d)
			835.4100 Aerobic soil metabolism	50310309	Sandy loam	PRT	72.23% (120 d)
					Sandy clay loam	PRT	61.57% (120 d)
					Silt loam	PRT	99.6% (120 d)
MAJOR (>10%) TRANSFORMATION PRODUCTS							
Unextractable residues	NA	NA	835.4100 Aerobic soil metabolism	50310309	Sandy loam	**77.01%** (32 d)	**76.18%** (120 d)
Carbon dioxide	Carbon dioxide **Formula:** CO_2 **MW:** 44 g/mol **SMILES:** C(=O)=O		835.4100 Aerobic soil metabolism	50310309	Sandy loam	**28.09%** (32 d)	**23.40%** (120 d)
					Sandy clay loam	**28.0%** (120 d)	**28.0%** (120 d)
					Silt loam	**31.19%** (120 d)	**31.19%** (120 d)

ND= means “not detected”. AR means “applied radioactivity”. MW means “molecular weight”. Bolded values are laboratory study values >10%AR.

Appendix B. Full Suite of Nicarbazin Ecotoxicity Data

Table B-1. Aquatic Toxicity Endpoints for Nicarbazin

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value in mg a.i./L (unless otherwise specified)	MRID or ECOTOX No./ Classification	Comments
Freshwater Fish (Surrogates for Vertebrates)					
Acute	DNC (98% a.i.)	Rainbow trout (*Onchorhynchus mykiss*)	96-h LC_{50} >0.069[1]	46416432 (Acceptable)	No mortality or sublethal effects were noted at the reported water solubility level for DNC.
	HDP (99.4% a.i.)		96-h LC_{50} >110[2] (practically non-toxic)	46416431 (Acceptable)	No mortality or sublethal effects were observed.
	DNC (98% a.i.)	Bluegill sunfish (*Lepomis macrochirus*)	96-h LC_{50} >0.072[3]	46416434 (Supplemental)	No mortality or sublethal effects were noted at the reported water solubility level for DNC. The test fish weight was lower than recommended, resulting in a reduction in study classification.
	HDP (99.4% a.i.)		96-h LC_{50} >122[4] (practically non-toxic)	46416433 (Supplemental)	No mortality or sublethal effects were observed. The test fish weight was lower than recommended, resulting in a reduction in study classification.
Chronic	No data submitted				
Estuarine/Marine Fish (Surrogates for Vertebrates)					
Acute	No data submitted				
Chronic	No data submitted				
Freshwater Invertebrates (Water-Column Exposure)					
Acute	DNC (98% a.i.)	Waterflea (*Daphnia magna*)	48-h LC_{50} >0.093[5]	46416436 (Acceptable)	Maximum mortality was ≤25% (0.064 mg a.i./L). Lethargy was observed in the 0.064 and 0.093 mg a.i./L test concentrations. The highest concentration was above reported water solubility level for DNC.
	HDP (99.4% a.i.)		48-h LC_{50} >107[6] (practically non-toxic)	46416435 (Acceptable)	No treatment related mortality or sublethal effects (≤5%).
Chronic	No data submitted				
Estuarine/Marine Invertebrates (Water-Column Exposure)					
Acute	No data submitted				
Chronic	No data submitted				
Freshwater Invertebrate (Sediment Exposure)					
Chronic	No data submitted				

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value in mg a.i./L (unless otherwise specified)	MRID or ECOTOX No./ Classification	Comments
Estuarine/ Marine Invertebrates (Sediment Exposure)					
Chronic	No data submitted				
Aquatic Plants and Algae					
Vascular	No data submitted				
Non-vascular	No data submitted				

[1] No mortality or sublethal effects were reported in the study. Low analytical recoveries (57.3-77.1% of nominal) were attributed to the fact that the nominal test concentration of 100 ppb a.i. (0.1 mg a.i./L) was in excess of the maximum solubility of the test material. The test was conducted at or above the limit of solubility for DNC in the test system. For DNC the aqueous solubility was 2.66 × 10-5 mg/L at pH5, 4.65 × 10-5 mg/L at pH 7; and 8.09 × 10-5 mg/L at pH 9. (MRID 46416411).

[2] No mortality or sublethal effects were reported in the study. For HDP the aqueous solubility was 19.0 mg/L at pH5; 19.3 mg/L at pH7, and 20.0 mg/L at pH 9 (MRID 46416411).

[3] No mortality or sublethal effects were reported in the study. Low analytical recoveries (63.0-77.8% of nominal) were attributed to the fact that the nominal test concentration of 100 ppb a.i. (0.1 mg a.i./L) was in excess of the maximum solubility of the test material. The test was conducted at or above the limit of solubility for DNC in the test system. For DNC the aqueous solubility was 2.66 × 10-5 mg/L at pH5, 4.65 × 10-5 mg/L at pH 7; and 8.09 × 10-5 mg/L at pH 9. (MRID 46416411). Test fish weight ranged from 0.35-0.76 g which was lower than recommended 0.5-5g; based on 10 negative control fish; the use of smaller than recommended fish affected the acceptability of the study.

[4] No mortality or sublethal effects were reported in the study. The test was conducted at or above the limit of solubility for DNC in the test system. Test fish weight ranged from 0.29-0.50 g which was lower than recommended 0.5-5g; based on 10 negative control fish; the use of smaller than recommended fish affected the acceptability of the study. For HDP the aqueous solubility was 19.0 mg/L at pH5; 19.3 mg/L at pH7, and 20.0 mg/L at pH 9 (MRID 46416411).

[5] At 48 hours 5, 25, 5, 0, and 5% mortality/immobilization daphnids was observed in the mean-measured 0.093, 0.064, 0.040, 0.027, and 0.017 mg a.i./L test concentrations, respectively; and there was 0 and 5% mortality/immobilization of daphnids in the negative and solvent controls, respectively. Sublethal effects (lethargy) was observed at 24 hours in 25, 29, and 42% of surviving daphnids in the 0.040, 0.064, and 0.093 mg a.i./L test concentrations, respectively; at 48 hours, lethargy was observed in 73 and 21% of surviving daphnids in the 0.064, and 0.093 mg a.i./L test concentrations, respectively. All other surviving daphnids in the other test concentrations at the remaining time points appeared normal. Analytical recoveries (78.1-139.2% of nominal); the nominal test concentration of 100 ppb a.i. (0.1 mg a.i./L) was in excess of the maximum solubility of the test material. For DNC the aqueous solubility was 2.66 × 10-5 mg/L at pH5, 4.65 × 10-5 mg/L at pH 7; and 8.09 × 10-5 mg/L at pH 9. (MRID 46416411).The test was conducted at or above the limit of solubility for DNC in the test system.

[6] At 48 hours, there was 5% mortality/immobilization (1 dead/immobilized) in the mean-measured 15 mg a.i./L test concentration, only; all other daphnids survived test duration (negative control and mean-measured 24, 39, 66, and 107 mg a.i./L test concentrations). After 48 hours of exposure, all surviving daphinds appeared normal in the negative control and mean-measured 15, 24, 39, and 107 mg a.i./L test concentrations. At 48 hours, one daphnid (5%, n=1/20) was observed to be lethargic in the mean-measured 66 mg a.i./L test concentration, only. For HDP the aqueous solubility was 19.0 mg/L at pH5; 19.3 mg/L at pH7, and 20.0 mg/L at pH 9 (MRID 46416411).

Table B-2. Terrestrial Toxicity for Nicarbazin

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Birds (Surrogates for Terrestrial Amphibians and Reptiles)					
Acute Oral	Nicarbazin complex (>99% a.i.)	Bobwhite Quail (*Colinus virginianus*)	14-days LD_{50} > 2250 mg a.i./kg-bw[1]	46416426 (Acceptable)	Practically non-toxic; No mortality or clinical signs of toxicity were observed.
Sub-acute dietary	Nicarbazin complex (>99% a.i.)	Mallard Duck (*Anas platyrhynchos*)	8-days LC_{50} = 3680 mg a.i./kg-diet[2]	46445302 (Acceptable)	Slightly toxic Sublethal effects included slight loss of coordination, a ruffled appearance, lower limb weakness, reduced reaction to external stimuli, loss of righting reflex, lethargy, gaping, wing droop, prostrate posture, and/or thinness. Some effects persisted through test termination.
Sub-acute dietary	Nicarbazin complex (>99% a.i.)	Bobwhite Quail (*C. virginianus*)	8-days LC_{50} >5720 mg a.i./kg-diet[3]	46416427 (Acceptable)	Practically non-toxic Sublethal effects were first observed between Days 5 and 6, and included wing droop, a ruffled appearance, and/or foot lesions (from picking).
Chronic (reproduction)	Nicarbazin (unknown % a.i.)	Broiler breeder chicken (*Gallus gallus domesticus*)	2-week Exposure NOAEC < 2 LOAEC = 2 mg/kg-diet (early embryonic mortality (43% at the LOAEC), most sensitive endpoint)[4] Post-exposure (2 weeks after exposure) NOAEC = 40 LOAEC >40 mg/kg-diet (no effects)	50310301[N] Supplemental (QUAL)	Other endpoints affected included fertility, and hatchability. All observed effects continued until the first week post-treatment, and by the second week post-treatment effects were comparable to control at all levels.
Mammals					
Acute Oral	Nicarbazin complex >95% a.i	Laboratory rat (*Rattus norvegicus*)	LD_{50} = >10,000 mg a.i./kg-bw[5] (unspecified sex)	46416413 (Acceptable)	Practically non-toxic

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Acute Inhalation	Nicarbazin complex >95% a.i	Laboratory rat (R. norvegicus)	4-hours LC_{50} > 0.147 mg/L[6] (M & F)	46416415 (Acceptable)	4-hour snout only; used 30% granulated pre-mix product All animals survived the test. Clinical signs included exaggerated breathing in female rats during exposure (as of 30 minutes) and all rats from 2 hours into exposure. All rats were normal by day 2.
90-day Oral	Nicarbazin Complex	Laboratory rat (*R. norvegicus*)	90-day NOAEL < 181 mg/kg-bw/d LOAEL = 181 mg/kg-bw/d	EFSA, 2010	Based on decreases in body weights, erythrocytes, hemoglobin, & hematocrit. Increases in blood urea nitrogen & plasma creatinine. Increases in several relative organ weights. Tubular degeneration in the kidney & degeneration in the seminiferous tubule (in males).
90-day Oral	DNC	Laboratory rat (*R. norvegicus*)	90-day NOAEL = 709 mg/kg-bw/d	EFSA, 2010	No effects were identified
Chronic (2-generation reproduction)	3:1 ratio of DNC:HDP	Laboratory rat (*R. norvegicus*)	2-generation NOAEL> 400 mg a.i./kg-bw/day	46416422 (Acceptable)	Reproductive: No effects (parental or reproductive effects) were identified.
Chronic Developmental	3:1 ratio of DNC:HDP		NOAEL = 200 LOAEL =600 mg a.i./kg-bw/day (maternal)	46416421 (Acceptable)	Developmental toxicity: Decreased fetal weights observed in the presence of maternal toxicity (decreased body weights in dams).
Terrestrial Invertebrates					
Acute contact (adult)	No data submitted				
Acute oral (adult)	No data submitted				
Chronic oral (adult)	No data submitted				

Study Type	Test Substance (% a.i.)	Test Species	Toxicity Value[1]	MRID or ECOTOX No./ Classification	Comments
Acute oral (larval)	No data submitted				
Chronic oral (larval)	No data submitted				
Foliage Residue	No data submitted				
Semi-field study or full field study)	No data submitted				
Terrestrial and Wetland Plants					
Seedling Emergence	No data submitted				
Vegetative Vigor	No data submitted				

[1] No mortality or clinical signs of toxicity were observed in any control or test group during the study, and no-treatment related effects on body weight or feed consumption were observed. Nicarbazin technical consists of equimolar quantities of two moieties, 4,4-'dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). The purity of both moieties was >99%; resulting in an overall purity for nicarbazin technical of >99% on a w:w basis.

[2] Mortality was 0% in the control, and ≤802 ppm a.i. treatment levels, 10% at the 1968 ppm a.i. level, and 80% at the 5720 ppm a.i. level. Treatment-related signs of toxicity were observed in birds from the 1968 and 5720 ppm a.i. treatment levels. Effects were first observed on Days 4 and 2, respectively, and included a slight loss of coordination, a ruffled appearance, lower limb weakness, reduced reaction to external stimuli, loss of righting reflex, lethargy, gaping, wing droop, prostrate posture, and/or thinness. Affected birds from the 1968 ppm a.i. group generally improved during the recovery period; however, a slight loss of coordination and lower limb weakness persisted in the two surviving birds from the 5720 ppm a.i. group through test termination. Dose-response effects on mean body weight gain were observed at the 802, 1968, and 5720 ppm a.i. treatment levels during the exposure period, and at the 5720 ppm a.i. during the recovery period. Body weight changes were the most significant endpoint – NOAEC = 344 ppm a.i. Feed consumption was reduced notably at the 1968 and 5720 ppm a.i. treatment levels during the exposure period. Nicarbazin technical consists of equimolar quantities of two moieties, 4,4-'dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). The purity of both moieties was >99%; resulting in an overall purity for nicarbazin technical of >99% on a w:w basis.

[3] No treatment-related mortalities occurred ≤802 ppm a.i. treatment levels. Single mortalities (10%) were observed at the 1968 and 5720 ppm a.i. treatment levels that may have been caused from exposure. Treatment signs of toxicity were also observed in the birds from the 1968 and 5720 ppm a.i. treatment levels. Effects were first observed between Days 5 and 6, and included wing droop, a ruffled appearance, and/or foot lesions (from picking). Dose-response effects on mean body weight gain were observed at the 802, 1968 and 5720 ppm a.i. treatment levels during the exposure period; with no differences during the recovery period. Body weight changes were the most significant endpoint – NOAEC = 344 ppm a.i. Feed consumption was reduced notably at the 5720 ppm a.i. level during the exposure period, but no apparent effects were observed at the lower treatment levels or at any level during the recovery period. Nicarbazin technical consists of equimolar quantities of two moieties, 4,4-'dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). The purity of both moieties was >99%; resulting in an overall purity for nicarbazin technical of >99% on a w:w basis.

[4] Avian Reproduction; (most sensitive endpoint: early embryonic mortality 43% at the LOAEC)

[5] Based on these results the test substance is EPA Toxicity Category IV according to HED.

[6] The acute (4-hour) rat inhalation study was conducted using 30% nicarbazin granulated premix. Dosing was via snout only, and particulate exposure was at an average maximum (gravimetric) concentration of 0.147 mg/L at an MMAD 8.2 µ, plus a control group. All animals survived the test; clinical signs of toxicity that were observed included exaggerated breathing in female rats during exposure as of 30 min and to all rats from 2 hours into exposure. Wet fur and exaggerated breathing were observed in all rats immediately following exposure; most/all

rats were normal by day 2. Female rats showed reduced mean body weight gain throughout the study. There were no gross lesions at necropsy. Based on this, the test substance is EPA Toxicity Category IV according to HED.

***Additional Avian Toxicity Data* (summarized from; DP328803; USEPA, 2007a)**

No guideline data have been submitted for avian reproduction (mallard, northern bobwhite), and that is a data gap. Besides the recently submitted chicken reproduction study (MRID 50310301), the registrant did submit several supplemental studies that provide some information on adverse reproductive effects in chickens (Table B-3). Based on these data, a NOAEC of 10 ppm is presumed due to adverse effects on egg hatching at 20 ppm. Some measurement endpoints required in guideline avian reproduction studies were not addressed (e. g., survival and growth of chicks) in these studies, which only addressed effects in chickens. Therefore, NOAEC has not been established. The data do, however, clearly demonstrate that low concentrations of nicarbazin fed in the diet will adversely impact avian reproduction. For example, significantly reduced egg production and hatchability of fertile eggs was reported at a dietary concentration of 25 ppm when fed to chickens for only four days, and eggshell pigmentation was reduced after only two days of feeding (Hughes *et al.,* 1991). These effects were even more pronounced at dietary concentrations of 50 and 100 ppm. Jones *et al.*, (1990a,b,c) reported negative impacts on egg hatchability in chickens fed 20 ppm nicarbazin in the diet for only 9 to 10 days in several studies. Ott *et al.,* (1956) reported reduced egg hatchability in chickens fed 50-700 ppm. These studies, as well as studies on other species of birds, are discussed in more detail below.

Table B-3. Adverse Reproductive Effects Reported in Chickens Exposed to Nicarbazin In Their Diet

Dietary level (ppm)	No. days treated	Significant adverse effects
Hughes et al. (1991)[1]:		
25	2	reduced eggshell pigmentation
	4	reduced egg production, hatchability, and eggshell pigmentation
	6	reduced egg production, egg weight, hatchability, and eggshell pigmentation
50	2	reduced hatchability and eggshell pigmentation
	4	reduced egg production and eggshell pigmentation
	6	egg production, hatchability, and eggshell pigmentation
100	2	reduced egg production, hatchability, and eggshell pigmentation
	4	reduced egg production, egg weight, fertility, hatchability, and eggshell pigmentation
	6	reduced egg production, egg weight, hatchability, and eggshell pigmentation
Jones et al. (1990a)[1]:		
10	10	None
20	10	reduced egg hatching after 9 days
50	10	reduced egg hatching after 5 days reduced eggshell pigmentation after 3 days; reduced to 0 by day 8
100	10	reduced egg hatching after 5 days; hatchability <1% after 7 days reduced egg production after 9 days reduced egg weight after 9 days reduced eggshell pigmentation after 3 days; reduced to 0 by day 6
Jones et al. (1990b)[1]:		
20	10	reduced egg hatching after 10 days

Dietary level (ppm)	No. days treated	Significant adverse effects
50	10	reduced egg hatching after 6 days
100	10	reduced egg hatching after 4 days
125	10	reduced egg hatching after 2 days eggshell pigmentation reduced to 0 after 2 days reduced egg production after 4 days
Jones et al. (1990c)[1]:		
20, 50, 100	10	reduced egg production and egg weight
125	10	reduced egg production after 1-2 days reduced egg weight after 8 days reduced eggshell thickness after 8 days
Ott et al. (1956)[2]: reproductive effects		
0	1-day-old to 32-36 wks of age	77% hatchability; brown eggs
50		32% hatchability; med. brown eggs
100		31% hatchability; light brown eggs
200		5% hatchability; brown-tinted eggs
400		13% hatchability; chalk-white eggs; decreased egg production; decreased egg wt.
700		0% hatchability

[1] published data submitted by the registrant (MRID No. 46497101)

[2] published data obtained from the literature

Information from Laboratory Studies

Chickens: As summarized in Table B-3 above, several studies have reported on the adverse effects of nicarbazin when fed to chickens at concentrations of 10 to 125 ppm in their diet for periods ranging from 2 to 10 days (Hughes *et al.,* 1991; Jones *et al.*, 1990a,b,c) and levels of 50 to 2000 ppm for periods from 3 to 36 weeks (Ott *et al.,* 1956). Most concentrations are considerably lower than the 5000 ppm OvoControl P food bait, which will be applied daily for up to 52 weeks. Endpoints evaluated in one or more of the studies included mortality, egg production, fertility, egg hatchability, egg weight, eggshell pigmentation, and depressed growth. The chicken information is summarized below.

Adverse effects on egg production, egg weight, egg hatchability, and eggshell pigmentation was found in chickens fed 25, 50, or 100 ppm nicarbazin for 2, 4, or 6 days at each level (Hughes *et al.,* 1991). At 25 ppm, eggshell pigmentation was affected after 2 days, egg production and hatchability after 4 days, and egg weight after 6 days. After only 2 days, egg hatchability and eggshell pigmentation were reduced at 50 ppm and 100 ppm and egg production, hatchability, and eggshell pigmentation were impacted at 100 ppm.

Jones *et al.*, (1990a) fed nicarbazin in the diet to chickens at levels of 10, 20, 50, and 100 ppm for 10 days. No significant adverse effects were observed at 10 ppm, but egg hatching was reduced after 9 days at 20 ppm. Egg hatching was reduced after only 5 days for birds fed 50 ppm, and eggshell pigmentation was reduced to 0 by day 8. Egg hatchability was <1% after 7 days of feeding on 100 ppm nicarbazin, eggshell pigmentation was reduced after 3 days, and egg production and egg weight were impacted after 9 days.

Jones *et al.*, (1990b,c) also fed nicarbazin to two varieties of chickens at levels of 20, 50, 100, and 125 ppm for 10 days and assessed effects on reproduction. Egg production, hatching, and egg weight was reduced at all levels of nicarbazin in the diet. At 125 ppm, egg production was reduced after only 1-2 days of feeding, eggshell pigmentation reduced to 0 after 2 days, egg production was reduced after 4 days, and egg weight and eggshell thickness was reduced after only 8 days.

Ott *et al.*, (1956) reported hatchability reduced to zero in birds fed 700 ppm nicarbazin from 1 day to 32-36 weeks of age, was decreased to about 10% of normal in birds fed 200 and 400 ppm and was impaired by about 60% at 50 or 100 ppm. The most striking and rapid effect of nicarbazin on reproduction was reduced eggshell pigmentation in hens fed 100 ppm and higher in their ration, and almost all shells were chalk white at 400 ppm. Decreased egg production and decreased egg weight was reported at 400 ppm. The potential impacts of reduced eggshell pigmentation are not known, but white eggs might be more visible and easily detected by avian, mammalian, and reptilian egg predators than would be dark or cryptically colored eggs normally laid by many bird species.

Effects of anticoccidial drugs on body weight and feed consumption of male broiler chicks were examined by Keshavarz and McDougald (1982). Nicarbazin was fed to chicks (1 to 4 weeks of age) at concentrations of 0, 125, 187.5, 250, 312.5, or 375 ppm for three weeks. Although no mortality was reported at 375 ppm nicarbazin in the diet, 7.5% mortality was observed at 312.5 ppm. Body weight was significantly lower than the control birds after one week of feeding at 312.5 and 375 ppm and at $\geq$187.5 ppm after 2 weeks of feeding. Feeding also was significantly depressed at $\geq$312.5 ppm.

Few data were found on nicarbazin levels in body tissues, but some metabolism data in chickens administered a 50 ppm [^{14}C]-DNC-nicarbazin diet for 5 consecutive days was cited in a review of nicarbazin by the European Food Safety Authority (EFSA 2003). The highest radioactivity was found in the liver, followed by the kidneys, fat, skin, and muscle, but residue levels were not reported. In another study, [^{14}C]-labelled on either the DNC or HDP moieties, at 125 ppm in the diet for 3 consecutive days, then sacrificed at 0, 5, 8, 11, 14, and 21 days after withdrawal. The original data were not available to EFSA (2003) but appeared to indicate that DNC residues are much higher and persistent, especially in the liver, than HDP which was not detectable 5 days after withdrawal. DNC residues were still measurable at a significant level after 21 days in liver (0.063 mg/kg wet wt) and muscle (0.074 mg/kg wet wt). No data were obtained on residue levels in skin and fat. Ott *et al.*, (1956) calculated that the carcass of a 4-5 lb. chicken would contain about 3-4 mg of DNC in the muscles and 0.5-1 mg of DNC in the liver immediately after feeding on 200 ppm nicarbazin for 12 weeks. Presumably, nicarbazin levels in body tissues of animals that eat 5000 ppm bait for up to 52 weeks would be higher due to both increased exposure and biomagnification. Whether those residues pose acute and reproductive/chronic risks to non-target animals is unknown.

***Pigeons*:** In a report translated from Italian and submitted by Innolytics to support the registration of nicarbazin, Martelli *et al.*, (1993) reported that nicarbazin was efficacious against pigeons in the laboratory. They fed nicarbazin to pigeons at 0, 50, 230, and 400 ppm (10 pairs per group) for the duration of the nesting period, which lasted about 120 days. According to the translation, "*. even at the lowest dose used in the trials (50 ppm), the overall fertility rate (live and active nestlings/expected nestlings) is visibly reduced by up to 33.3% (average value for three nesting cycles)*." At 400 ppm, the overall fertility rate was 0%. These findings are surprising in light of the fact that the registrant is proposing a 5000 ppm bait for pigeons.

A 2006 study conducted by USDA/APHIS National Wildlife Research Center tested the effects of 5000 ppm test on pigeons (Avery 2006, MRID 46820701). Pairs of pigeons were observed during pretreatment, treatment, and recovery periods to determine the effect of 5000 ppm bait on hatchability, DNC levels in the blood and unhatched eggs, and surviving chick weight. Pairs of pigeons were fed up to 40g (8x the minimum rate on the label) of bait daily during the treatment period. DNC levels in the blood of females and daily food consumption of pairs were related to DNC levels in their unhatched eggs. No difference between mean 14-day-old chick body weights was observed between chicks hatched from the pretreatment and treatment periods. A 59% decrease in hatch was observed between the pretreatment and treatment period. Estimated daily bait consumption ranged from 13.6 to 25.3 g per pair; thus the reduction in hatchability observed in this study was achieved with 2.7 to 5.1 times the minimum rate suggested on the proposed OvoControl P label. Further, three pairs demonstrated reduced productivity during the recovery phase, two of which laid eggs containing DNC. This result demonstrates the potential bioaccumulation of DNC and its residual effects that can occur after feeding ceases.

In contrast to the report of Martelli *et al.*, (1993), Elder (1964) reported that nicarbazin had no adverse effects on pigeon reproduction. He tested nicarbazin as one of several possible oral contraceptives for nuisance birds. Nicarbazin was tested against pigeons at 100 ppm (0.01%) and 1000 ppm (0.1%) active ingredient in the diet for 19 days. At 100 ppm, all females survived and continued to lay fertile eggs at the rate of 2 clutches per month for the following 3 months. However, at 1000 ppm in the diet, 8 of the 20 pigeons died, and survivors continued to lay fertile eggs when returned to normal feed after 6 days. The author concluded that "*Again we must eliminate a compound of great promise as having no effect on pigeons, even when they received an approximate LD_{50} dose*."

Other birds: Some information is available on nicarbazin levels in plasma and eggs and its effects on egg hatching in other birds, including chickens, mallards, quail, and Canada geese. Most of this work was conducted by the USDA/APHIS National Wildlife Research Center. Collectively, these studies show that the Canada goose absorbs less nicarbazin from its food than do other birds. Consequently, adverse reproductive effects actually may be considerably more severe in exposed non-target species than in the target species. Adverse effects occurred at lower concentrations and for a shorter exposure time than that possible from applying the OvoControl P nicarbazin food bait for up to 13 weeks. One study demonstrated that "*. . . DNC levels in goose eggs of >2 µg/ml are required to render eggs nonviable and that daily doses of*

250 to 500 ppm in the diet are sufficient to achieve this level." Another study with mallards found that 500 ppm for 14 days resulted in only 0-15% hatchability of eggs laid. These studies are summarized below.

Quail: Miller *et al.*, (2005) examined the reproductive effects of nicarbazin in Coturnix quail (*Coturnix coturnix*). When fed 125 ppm nicarbazin in the diet for 4 weeks, peak egg nicarbazin levels of 2.7 µg/g occurred in week 2 of treatment. During the 4-week treatment phase, overall treated egg hatchability was 11.5% (3 eggs hatched of 26 incubated), and 0% hatchability was reached by week 4.

The dietary guideline toxicity test using the northern bobwhite established an LC_{50} >5620 ppm (see Table C-2). No treatment related mortality occurred ≤800 ppm, however one incidental mortality occurred at the 320 ppm test level. Additionally, single mortalities (10%) were observed at the 2000 and 5625 ppm test levels. Signs of toxicity were observed by Days 5 and 6 and included wing droop, ruffled appearance, and/or foot lesions. Gallinaceous birds (and probably other birds) might be at risk if they feed on the 5000 ppm bait for periods of several days or more during a baiting program that may last for many weeks of daily baiting.

***Mallards*:** Yoder (2005) evaluated the mechanism of action for nicarbazin and assessment of its use as an avian contraceptive at treatment levels of 0, 125, 250, and 500 ppm for 14 days. DNC plasma and egg levels in mallards peaked on days 10-12, and peak reduction in hatchability occurred beginning at day 10. Hatchability decreased with increasing nicarbazin concentration and was reduced to 0-15% at 500 ppm (Table C-4).

Table B-4. Peak DNC Concentrations in Plasma and Eggs and Hatchability of Eggs in Nicarbazin-treated Mallards (from Yoder 2005)

Treatment (ppm)	No. days treated	Peak plasma DNC (µg/ml)	Peak egg DNC (µg/g)	Hatchability (%)
0	14	0	0	65-75
125	14	1.5-1.7	3.0-3.2	45-65
250	14	1.7-1.9	4.8-5.7	20-25
500	14	3.8-4.0	6.9-8.8	0-15

The LC_{50} of 3680 ppm established in the dietary toxicity study with the mallard (see **Table B-2**) categorizes nicarbazin as slightly toxic. However, 10% mortality occurred at 1968 ppm and 80% mortality at 5720 ppm when nicarbazin was fed in the diet for 5 days. Treatment-related signs of toxicity were evident by Day 2, including slight loss of coordination, ruffled appearance, lower limb weakness, reduced reaction to external stimuli, loss of righting reflex, lethargy, gaping, wing droop, prostrate posture, and/or thinness; reduced food consumption; reduced body-weight gain. These findings suggest that mallards (and probably other waterfowl) feeding on the 5000 ppm bait for a day or more will be at risk.

Canada goose: The dose-efficacy of nicarbazin-treated feed for reducing the reproductive success of Canada geese was examined by Vercauteren (2005). Penned geese were fed nicarbazin at 0, 125, 250, or 500 ppm from the onset of breeding until they initiated incubation. Preliminary results indicate that DNC levels in goose eggs of >2 µg/ml are required to render eggs nonviable and that daily doses of 250 to 500 ppm in the diet are sufficient to achieve this level.

Miller (2005) orally gavaged 8.4 mg/kg nicarbazin daily for 8 days to Canada geese as well as mallards and chickens. Chickens absorbed more nicarbazin from the gut than did mallards, and mallards absorbed more than geese. Peak plasma levels occurred at days 6-8 and were 2.8 µg/ml for chickens, 2.3 µg/ml for mallards, and 1.3 µg/ml for geese. The author concludes that the dose required to exert an effect on hatchability and egg production of geese will be significantly higher than the dose required to have such an effect in the chicken due to this plasma difference.

Other avian information: Nicarbazin has been shown to exacerbate the effects of heat stress (McDougald and McQuistion 1980, Keshavarz and McDougald 1981, Beers *et al.*, 1989, Wiernusz and Teeter 1995). For example, McDougald and McQuistion (1980) examined the relationship of nicarbazin to heat stress mortality in broilers in a replicated floor-pen experiment during a period of hot weather in Georgia. During the 8-week study, mortality averaged 36% in nicarbazin-medicated birds versus 6% mortality in unmedicated birds or those medicated with monensin or lasalocid. Most of the deaths coincided with three periods of hot weather and were attributed to heat stress. In another study in which 125 ppm nicarbazin was fed to broiler chickens, 11 heat-related mortality episodes were recorded in the first experiment. In a second experiment, in which the birds were exposed to a constant 37.8°C, nicarbazin-treated birds suffered more severely from heat stress in a short period of time than did unmedicated birds. No data are available to assess the potential acute and reproductive affects to non-target birds exposed to the 5000 ppm bait and subjected to periods of hot weather, but EFED believes impacts could occur and might possibly be severe in some situations.

Appendix C. ECOSAR OUTPUTS

DNC

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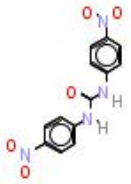
Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
		[H]N(C(N(C1=CC=C(N([O])[O])C=C1)[H])=O)C2=CC=C(N([O])[O])C=C2

Structure


Details	
MolWt	305.28
Selected LogKow	0.96
Selected Water Solubility (mg/L)	1696.57
Selected Melting Point (°C)	
Estimated LogKow	0.96
Estimated Water Solubility (mg/L)	1696.57
Measured LogKow	
Measured Water Solubility (mg/L)	
Measured Melting Point (°C)	

Class Results:	

Substituted Ureas

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	605.85	5	
Daphnid	48h	LC50	353	5	
Green Algae	96h	EC50	1.04	6.4	
Fish		ChV	12.85	8	
Daphnid		ChV	14.48	8	

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Green Algae		ChV	0.37	8	
Fish (SW)	96h	LC50	83.24	5	
Mysid (SW)	96h	LC50	95.99	5	
Fish (SW)		ChV	3.16	8	
Mysid (SW)		ChV	28.25	8	

HDP

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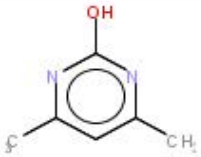
Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
108792	2(1H)-Pyrimidinone, 4,6-dimethyl-	n(c(cc(n1)C)C)c1O

Structure



Details	
MolWt	124.14
Selected LogKow	1.3
Selected Water Solubility (mg/L)	7447.86
Selected Melting Point (°C)	
Estimated LogKow	1.3
Estimated Water Solubility (mg/L)	7447.86
Measured LogKow	
Measured Water Solubility (mg/L)	
Measured Melting Point (°C)	

Class Results:	

Phenols

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	50.88	7	
Daphnid	48h	LC50	16.1	7	
Green Algae	96h	EC50	4.36	6.4	
Fish		ChV	4.7	8	
Daphnid		ChV	1.56	8	
Green Algae		ChV	7.85	8	
Fish (SW)	96h	LC50	62.52	7	

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Mysid (SW)	48h	LC50	14.22	7	
Green Algae (SW)	96h	LC50	249.01	6.4	
Lemna gibba	7d	EC50	61.9	6.4	

Appendix D. Endocrine Disruptor Screening Program (EDSP)

As required by FIFRA and the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA reviews numerous studies to assess potential adverse outcomes from exposure to chemicals. Collectively, these studies include acute, subchronic and chronic toxicity, including assessments of carcinogenicity, neurotoxicity, developmental, reproductive, and general or systemic toxicity. These studies include endpoints which may be susceptible to endocrine influence, including effects on endocrine target organ histopathology, organ weights, estrus cyclicity, sexual maturation, fertility, pregnancy rates, reproductive loss, and sex ratios in offspring. For ecological hazard assessments, EPA evaluates acute tests and chronic studies that assess growth, developmental and reproductive effects in different taxonomic groups. As part of the Draft Ecological Risk Assessment for Registration Review, EPA reviewed these data and selected the most sensitive endpoints for relevant risk assessment scenarios from the existing hazard database. However, as required by FFDCA section 408(p), nicarbazin is subject to the endocrine screening part of the Endocrine Disruptor Screening Program (EDSP).

EPA has developed the EDSP to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Under FFDCA section 408(p), the Agency must screen all pesticide chemicals. Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. A second list of chemicals identified for EDSP screening was published on June 14, 2013[5] and includes some pesticides scheduled for registration review and chemicals found in water. Neither of these lists should be construed as a list of known or likely endocrine disruptors. Nicarbazin is not on List 1. For further information on the status of the EDSP, the policies and procedures, the lists of chemicals, future lists, the test guidelines and Tier 1 screening battery, please visit our website[6].

[5] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0477-0074 for the final second list of chemicals.

[6] Available: http://www.epa.gov/endo/