Intorre L, et al., J Anim Res Vet Sci 2023, 7: 049
DOI: 10.24966/ARVS-3751/100049

HSOA Journal of
Animal Research and Veterinary Science

Research Article

Safety of Nicarbazin in Raptors in Relation to Pigeon Eradication Programs

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Abstract

The anticoccidial drug nicarbazin, mixed at a concentration of 800 mg/kg with corn kernels, is marked as Ovistop® and R-12® for the fertility control of pigeons. Nicarbazin is considered "practically non-toxic" when fed to birds and mammals, and the only potential adverse effect in non-target birds is a reduction in egg hatchability. Although there are no reports of adverse reactions in non-target animals including raptors in pharmacovigilance nor in toxicovigilance, in this paper the potential pathways of exposure were evaluated together with the risk of nicarbazin for European birds of prey living in the sites where nicarbazin could be used for the fertility control of pigeons. While primary exposure to nicarbazin was excluded, two possible pathways of secondary exposure were considered: by consuming undigested prey gut content or by preying on pigeons and feeding on their meat. From a toxicological point of view, the margins of exposure indicate that any potential secondary nicarbazin exposure of birds of prey is unlikely to lead to any acute or chronic toxicological risks. From a pharmacological point of view, nicarbazin does not constitute a risk for the reduction in egg hatchability of birds of prey. To have pharmacological effects, nicarbazin requires adequate exposure both in terms of dose and duration of at least five consecutive days throughout the entire nesting season, while the contraceptive effect is completely reversible after the treatment is withdrawn. The combination of these requirements makes it extremely unlikely that non-target pharmacological effects will occur. Considering that any potential exposure that birds of prey might suffer is well below the dosage (in terms of dose and duration of exposure) necessary for any toxicological or pharmacological effects, in raptors there is no risk from secondary exposure to nicarbazin in relation to pigeon eradication programs.

Keywords: Nicarbazin; Pigeon fertility control; Raptor safety

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Citation: Intorre L, De Marchi L, Gelati A, Colla P, Ferraresi M, et al. (2023) Safety of Nicarbazin in Raptors in Relation to Pigeon Eradication Programs. J Anim Res Vet Sci 7: 049.

Received: July 26, 2023; **Accepted:** August 04, 2023; **Published:** August 10, 2023

Introduction

The feral pigeon has adapted well to the urban environment, and an increase in overpopulated pigeon colonies has increased their economic, environmental and social impacts. Methods to mitigate these impacts include culling, toxicants and non-lethal methods such as fertility control. Although lethal control methods, including toxicants, are increasingly unacceptable to the general public, contraception is considered a humane tool to mitigate the impacts of pigeons [1,2].

Nicarbazin (NCZ) is one of the most common anticoccidial drugs used as a feed additive in poultry and other animal industry under the EU Additives Directive 70/524/EEC and is the only oral contraceptive in free-living birds. While NCZ has no negative effects on reproduction in the poultry industry, feeding NCZ to laying birds results in reductions in hatchability and egg production, and NCZ has proven to be a successful fertility control method for conflictive pigeon colonies in Italy, Spain and USA [3-7].

Two veterinary medicinal products (Ovistop®, ACME Drugs S.r.l., Italy and R-12®, ACME S.r.l., Italy) containing NCZ at 800 mg/kg, mixed with corn kernels and protected by stearic acid and dimethicone are available for the fertility control of pigeons [1,6]. The daily treatment rate of pigeons (average body weight = 300 gr) is 6.5-8 mg of NCZ corresponding to a maximum of 25 mg/kg bw, given in a daily single dose throughout the entire egg-laying period. The product is administered with an automatic feeder or hand broadcast [6]. As with any other substance placed into the environment, the use of NCZ raises issues regarding the health and safety of non-target animals including raptors, which are predatory birds positioned on the top of the food chain. Poisoning events in raptors have been reported from feeding on rodents exposed to anticoagulant rodenticides and carbamate and organophosphate insecticides, ingestion of poison baits used to control predators and feeding on domestic livestock carcasses treated with veterinary pharmaceuticals [8-10]. In contrast, there are no reports either in pharmacovigilance or in toxicovigilance of adverse reactions in raptors to the use of NCZ to control the fertility of pigeons.

The aim of the present paper is to evaluate the risk of NCZ for European birds of prey, considering the potential pathways of exposure to NCZ following its use for the fertility control of pigeons.

Methods

NCZ pharmacology

NCZ is an equimolecular complex of 4,4'-dinitrocarbanilide (DNC), the pharmacologically active component, and 2-hydroxy-4,6-dimethylpyrimidine (HDP) (specification 70% DNC, 30.0 % HDP) [11]. NCZ prevents embryo development, disrupting the vitelline membrane between the egg albumen and the yolk. Birds have to absorb NCZ continuously before (for 5-7 days) and during egg-laying to obtain contraceptive effects, while single or intermittent doses will not affect hatchability. The contraceptive effect is completely reversible, and the bird returns to normal reproduction within five days after the treatment is withdrawn [12]. After ingestion, nicarbazin splits into its two components (HDP and DNC), which undergo independent pharmacokinetics and metabolism. DNC alone is very poorly absorbed, and for absorption and to achieve a contraceptive blood level, the HDP component is required in a 1:1 molecular ratio as in nicarbazin. DNC is unable to re-complex with HDP and when administered alone or as a mixture of DNC+HDP, it shows a bioavailability that is 33 to 65 times lower than when administered as NCZ [4,5,13]. Residue decline rapidly after NCZ withdrawal [5,14]. In the chicken, which is the species with the highest NCZ absorption rate, after one-day withdrawal, concentrations of total residues (expressed as mg equivalent DNC/kg wet tissue) following administration of 125 mg [14C]-DNC-nicarbazin/kg feed for seven consecutive days were as follows (Table 1):

	One day withdrawal
Liver	27.797
Kidney	16.776
Muscle	4.431
Skin/fat	5.122

Table 1: Concentrations of total residues in different tissues after NCZ withdrawal expressed as mg equivalent DNC/kg wet tissue.

On this basis, an average NCZ tissue residue concentration of 13.5 ppm can be calculated after a one-day withdrawal. DNC and acetyl-DNC were the major residual compounds in all tissues, accompanied by a number of minor metabolites, each representing less than 10 %. DNC and its metabolites are mainly excreted in the feces (46 % unchanged DNC), while HDP appears in the urine, 90% as unchanged HDP) [4,5,13].

NCZ toxicology

NCZ is considered "practically non-toxic" when fed to birds and mammals.Toxicity data included in the WHO-FAO Joint Expert Committee on Feed Additives (JECFA) indicate that the acute toxicity of NCZ is very low: LD_{50} in the quail, which is the most sensitive bird, is > 2,250 mg/kg bw [15]. NCZ is considered practically non-toxic also in mammals and other species: LD_{50} in mice and rats greater than 10,000 mg/kg [15]. Repeated toxicity studies indicated NOEL between 200 mg/kg and 400 mg/kg in 2-year rat and dog studies, rat reproduction and teratology studies [15]. On this basis, the only potential adverse effect in non-target birds following secondary exposure to NCZ is considered the reduction in egg hatchability.

Exposure of birds of prey to NCZ

Birds of prey, commonly known as raptors, include vultures, eagles, hawks, kites, harriers, falcons, osprey and owls. Birds of prey are classified into two orders: Falconiformes and Strigiformes [16] the order of Falconiformes comprises five families (Accipitridae, Cathartidae, Falconidae, Pandionidae, Sagittariidae) which constitute more than 500 species of diurnal birds of prey, including hawks, eagles, vultures and falcons; the order of Strigiformes, which includes more than 200 species, comprises two families: Strigidae (typical owls) and Tytonidae (Barn- and Bay-owl). The eating habits of species of bird of prey living in the sites where NCZ is used for the fertility control of the pigeon population mean that the majority are unlikely to be exposed to NCZ. Most raptor species prey predominantly on mammals, especially rodent species. Mammals constitute 60–80% of the diets of barn owls, kestrels, Eurasian eagle-owls and red kites. Eurasian eagle-owls occasionally prey on larger mammals (e.g., hares, foxes, and deer) and red kites sometimes feed on hares, rabbits, and as carrion, foxes and deer [17-18]. Raptors also prey on other animals, such as birds, reptiles, amphibians, fish, and invertebrates. Birds constitute part of the diets in Eurasian sparrowhawks, golden eagles and red kites. In addition, the Eurasian eagle owls occasionally prey on other raptors (e.g., buzzards, falcons, tawny owls, and long-eared owls). Part of the diets of Eurasian eagle owls and bald eagles also consists of amphibians and fish, respectively. Invertebrates include some of the prey of moreporks, little owls and kestrels [17-18]. The amount of food a bird of prey ingests varies depending on many factors, including the species, weight, and sex of the bird. Smaller-sized birds have a greater surface area to body weight ratio than larger-sized birds, which results in greater heat loss. To compensate and maintain their metabolism and body temperature, smaller birds consume proportionally more food than larger birds which require a significantly lower percentage of their body mass as a daily food intake [16-18]. The daily food intake in birds of prey based on their body mass can be calculated as follows (Table 2):

Body mass	Daily food intake
up to 500 gr	25% of body mass
500-1200 gr	15% of body mass
>1,200 gr	5% of body mass

Table 2: Calculation of daily food intake in birds of prey.

Species, prey preferences and daily food intakes of birds of prey living in the sites where NCZ could be used and that potentially can prey on or feed on urban pigeon meat [19-20], are reported below (Table 3):

Order Falconiformes		
Family	***Genus***	***Species***
Accipitridae	Buzzard	Eurasian buzzard (*Buteo buteo*) Weight 700-1000 gr Diet: mainly small mammals, reptiles, amphibians, large insects, carrion. In cultivated areas frequented by pigeons it may come across a carrion or a dying pigeon that cannot escape Daily food intake (15% of body mass): 150 gr
	Harrier	Hen harrier (*Circus cyaneus*) Weight ♂ 250-400 gr, ♀ 300-570 gr Diet: especially juveniles and adults of small passerines, small rodents, reptiles and amphibians. It can attempt an attack on a stationary pigeon or a chase in flight, but it is notably slower than the pigeon Daily food intake (25% of body mass): up to 142 gr

		Montagu's harrier (*Circus pygarus*) Weight ♂ 230-300 gr, ♀ 330-430 gr Diet: especially juveniles and adults of small passerines, small rodents, reptiles and amphibians. It can attempt an attack on a stationary pigeon or a chase in flight, but it is notably slower than the pigeon Daily food intake (25% of body mass): 107 gr
		Western marsh-harrier (*Circus aeruginosus*) Weight ♂ 500-700 gr, ♀ 600-800 gr Diet: small mammals, nestlings and eggs of aquatic birds, amphibians, reptiles, injured or distressed birds. It can attempt an attack on pigeon that is stationary on the ground or a chase in flight, but it is notably slower than the pigeon Daily food intake (15% of body mass): 120 gr
	Hawk	Eurasian sparrowhawk (*Accipiter nisus*) Weight ♂ 100-170 gr, ♀ 200-350 gr Diet: feeds on birds, including pigeons. Occasionally, when it frequents urban environments, it may prey on pigeons that are stationary on the ground Daily food intake (25% of body mass): 87 gr
		Northern goshawk (*Accipiter gentilis*) Weight ♂ 580-830 gr, ♀1300 gr Diet: it preys on birds such as partridges, wood pigeons, corvids, mammals up to the size of a hare. The goshawk is an excellent predator and pigeons are not excluded from being captured, especially during the winter months when it also approaches anthropized areas. It may come into contact with pigeons and prey on them Daily food intake (15% of body mass): 195 gr
	Kites	Black kite (*Milvus migrans*) Weight 700-900 gr Diet: any food of animal origin, mainly fish and small animals, insects, small birds, and fish carrion. Occasionally it can feed on young pigeons in difficulty or their carrion Daily food intake (15% of body mass): 135 gr
		Red kite (*Milvus milvus*) Weight 900-1200 gr Diet: small mammals, snakes, lizards, fish, frogs, sick or injured small animals, even flightless birds, carrion, placentas. Occasionally it feeds on young pigeons in difficulty or their carrion Daily food intake (15% of body mass): 180 gr
Falconidae	Falcon	Lanner falcon (*Falco biarmicus*) Weight ♂ 500 gr; ♀ 900 gr Diet: mainly birds up to the size of the wood pigeon. Potential predator of the urban pigeon, but the risk of predation is practically nil given that it is a rare species and due to the environment where it lives (cliffs and rocky overhangs) Daily food intake (15% of body mass): 135 gr
		Peregrine falcon (*Falco peregrinus*) Weight ♂ 500-680 gr, ♀ 800-1200 gr. Diet: mainly birds that it catches in flight. The peregrine falcon may hunt pigeons it catches in flight Daily food intake (15% of body mass): 180 gr
Order Strigiformes		
Family	***Genus***	***Species***
Striginae	Typical owls	Eurasian eagle-owl (*Bubo bubo*) Weight ♂ 1.5-3 kg, ♀ 1.8-4.2 kg Diet: eagle owls are large predatory birds that prey on prey ranging from the size of a beetle to a young roe deer. In urban/suburban environments it may potentially prey on a pigeon; Daily food intake (5% of body mass): 210 gr
		Northern long-eared owl (*Asio otus*) Weight ♂ 207-330 gr, ♀ 235-435 gg Diet: Microtus, Soricidae, small birds, large insects. Low risk of pigeon predation Daily food intake (25% of body mass): 109 gr
		Tawny owl (*Strix aluco*) Weight ♂ 415-650 gr, ♀ 580-830 gg Diet: small insects, rodents, birds, amphibians, reptiles. Possible risk of pigeon predation Daily food intake (15% of body mass): 124 gr
Tytonidae	Barn and Bay owl	Common barn owl (*Tyto alba*) Weight 290-370 gr Diet: small mammals, even rats; small birds, reptiles, amphibians. It needs about 95 g of food per day and can potentially prey on pigeons. Low risk of pigeon predation. Daily food intake (25% of body mass): 92 gr

Table 3: Species, prey preferences and daily food intakes of birds of prey that live in sites where NCZ may be used.

Results and Discussion

Pathways of exposure to NCZ

The primary exposure to NCZ can be excluded because birds of prey will not consume corn grain-based products (carrier in Ovistop and R-12) and it is eaten in locations where birds of prey are typically not found,

There seem to be two possible pathways of secondary exposure (E_1, E_2) by which birds of prey may potentially be exposed to NCZ:

- E_1: when they consume undigested prey gut content.
- E_2: when they prey on pigeons and feed on their meat.

Secondary exposure to undigested NCZ

It is very unlikely for a predator to consume undigested NCZ. However, the consumption of NCZ through undigested prey gut content could also be considered a means of exposure.

In this scenario, on the basis of the maximum pigeon daily dose of 8 mg of NCZ, the estimated exposure (E_1) to undigested NCZ is calculated as follows:

$$E_1 = \frac{8\text{mg}}{\text{bird bw}}$$

For example, for the red kite (*Milvus milvus*), considering a bw of 1.2 kg, the equation is as follows:

$$E_1 \text{ (red kite)} = \frac{8 \text{ mg}}{1.2 \text{ kg}} = 6.7 \text{ mg/kg bw}$$

On this basis, the maximum estimated E_1 to NCZ of the relevant birds of prey, if they consume undigested NCZ in a dosed pigeon, are as follows (Table 4):

Species	*Body weight (kg)*	*E1 (mg/kg)*
Eurasian buzzard (*Buteo buteo*)	1.00	8.0
Hen harrier (*Circus cyaneus*)	0.57	14.0
Montagu's harrier (*Circus pygargus*)	0.43	18.6
Western marsh-harrier (*Circus aeruginosus*)	0.80	10.0
Eurasian sparrowhawk (*Accipiter nisus*)	0.35	22.9

Northern goshawk (*Accipiter gentilis*)	1.30	6.2
Black kite (*Milvus migrans*)	0.90	8.9
Red Kite (*Milvus milvus*)	1.20	6.7
Lanner falcon (*Falco biarmicus*)	0.90	8.9
Peregrine falcon (*Falco peregrinus*)	1.20	6.7
Eurasian eagle-owl (*Bubo bubo*)	4.20	1.9
Northern long-eared owl (*Asio otus*)	0.44	18.4
Tawny owl (*Strix aluco*)	0.83	9.6
Common barn Owl (*Tyto alba*)	0.37	21.6
Range	0.35-4.20	1.9-22.9

Table 4 : Estimated exposures to undigested NCZ of different species of birds of prey.

In this scenario, Eurasian sparrowhawks are likely to be the most exposed to 22.9 mg NCZ/kg bw.

Secondary exposure by pigeon predation and feeding

Birds of prey are potentially exposed to nicarbazin when they prey on treated pigeons. Assuming a nonrealistic scenario that the entire daily NCZ dose consumed by a pigeon is available as residue, the average residue concentration (R) in the pigeon carcass is 25 ppm (worst-case scenario: NCZ residue concentration twice that extrapolated from an experimental depletion study on chickens). Considering that DNC bioavailability in the pigeon carcass is reduced by at least 33 times after ingestion and absorption, and assuming that the entire daily food intake consists exclusively of pigeon meat, the E_2 of predatory birds to nicarbazin is calculated as follows:

$$E_2 = \frac{R}{33} \times \frac{\text{bird daily food intake}}{\text{bird bw}}$$

For example, for the red kite (*Milvus milvus*), considering a bw of 1.2 kg and a daily food intake of 180 gr of pigeon carcass (15% bw) containing 25 ppm of NCZ residue, the equation is as follows:

$$E_2 \text{ (Red Kite)} = \frac{25 \text{ mg/kg of NCZ}}{33} \times \frac{180 \text{ g}}{1.2 \text{ kg}} = 0.11 \text{ mg/kg bw}$$

On this basis, the estimated secondary exposures (E_2) to NCZ of the relevant birds of prey when they prey on treated pigeons and feed on its meat are as follows (Table 5):

Species	*Body weight (kg)*	*Daily food intake (gr)*	*E2 (mg/kg)*
Eurasian buzzard (*Buteo buteo*)	1.00	150.0	0.11
Hen harrier (*Circus cyaneus*)	0.57	142.5	0.19
Montagu's harrier (*Circus pygargus*)	0.43	107.5	0.19
Western marsh-harrier (*Circus aeruginosus*)	0.80	120.0	0.11
Eurasian sparrowhawk (*Accipiter nisus*)	0.35	87.5	0.19
Northern goshawk (*Accipiter gentilis*)	1.30	195.0	0.11
Black kite (*Milvus migrans*)	0.90	135.0	0.11
Red Kite (*Milvus milvus*)	1.20	180.0	0.11
Lanner falcon (*Falco biarmicus*)	0.90	135.0	0.11
Peregrine falcon (*Falco peregrinus*)	1.20	180.0	0.11
Eurasian eagle-owl (*Bubo bubo*)	4.20	210.0	0.04
Northern long-eared owl (*Asio otus*)	0.44	108.8	0.19
Tawny owl (*Strix aluco*)	0.83	124.5	0.11
Common barn owl (*Tyto alba*)	0.37	92.5	0.19
Range	0.35-4.20	87.5-180	0.04-0.19

Table 5 : Estimated exposures to NCZ of different species of birds of prey by pigeon predation and feeding.

In this scenario, smaller birds (i.e., hen harrier, Montagu's harrier, Eurasian sparrowhawk, Northern long-eared owl, common barn owl) are likely to be the most exposed to 0.19 mg NCZ/kg bw to because they eat proportionately more food than larger species such as the Eurasian eagle-owl *(Bubo bubo*).

Calculation of the Margins of Exposure (MoE)

From a toxicological point of view, the $MoE_{(LD)}$ expressed as the ratio of the lowest LD_{50} *vs* the highest E_1 and E_2 predatory bird exposures and the $MoE_{(NOEL)}$ expressed as the ratio of the lowest toxicological NOEL *vs* the highest E_1 and E_2 predatory bird exposures, are:

- $MoE_{(LD)}$ = 98 and 11,842 for E_1 and E_2, respectively
- $MoE_{(NOEL)}$ = 9 and 1,052 for E_1 and E_2, respectively

From a pharmacological point of view, the $MoE_{(Pharm)}$ expressed as the ratio of the contraceptive dose *vs* the highest E_1 and E_2 predatory bird exposures would be:

- $MoE_{(Pharm)}$ = 1.1 and 132 for E_1 and E_2, respectively

It follows that any potential secondary NCZ exposure of birds of prey is generally acceptable in terms of both acute and chronic toxicological risks. Furthermore, the consumption of the undigested NCZ does not constitute a risk in terms of pharmacological effects because NCZ requires a sufficiently high exposure both in terms of dose and duration of treatment. In fact, a single dose does not lead to a pharmacological effect and the non-target bird would need to ingest the product for at least five consecutive days for a contraceptive effect. Furthermore, non-target birds would need to consume NCZ throughout their entire nesting season and the contraceptive effect would be completely reversible as the bird returns to normal reproduction within five days after the treatment is withdrawn. The combination of these requirements makes it extremely unlikely that non-target pharmacological effects will occur.

Conclusion

While primary raptor exposure by direct consumption of the NCZ product can be excluded, raptors could be potentially exposed to NCZ both by consuming undigested gut content of treated pigeons (E_1) or by preying on treated pigeons and feeding on the meat (E_2). However, in both cases, the risk of adverse effects on nontarget birds of prey is extraordinarily low, and any potential exposure is estimated to be well below the dosage necessary to have any toxicological or pharmacological effects. There are thus no risk from secondary exposure to NCZ in raptors in relation to pigeon eradication programs.

Ethical Approval

The study does not require Ethics Committee approval.

Funding

This research received no external funding.

Competing Interest

The authors declare no conflict of interest.

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