E

CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda item 8.2 **CX/RVDF 26/28/10**

January 2026

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Twenty-eighth Session
23–27 March 2026

Minneapolis, Minnesota, United States of America

ESTABLISHMENT OF ACTION LEVELS FOR RESIDUES OF NICARBAZIN AND LASALOCID IN CHICKEN EGGS DUE TO UNAVOIDABLE AND UNINTENTIONAL CARRYOVER IN FEED

(At Step 4)

(Prepared by the Electronic Working Group chaired by Canada and co-chaired by Australia and the United States of America)

Codex members and observers wishing to submit comments on the action levels for residues of nicarbazin and lasalocid in chicken eggs, as presented in Appendix I, should do so as instructed in CL 2026/14-RVDF available on the Codex webpage/Circular Letters[1] or CCRVDF/Related Circular Letters[2]

INTRODUCTION

1. The 27th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF17, 2024), agreed to develop a complementary approach to address residues of veterinary drugs in food caused by unavoidable and unintentional carryover in feed, by:

   i. establishing Codex Action Levels; and

   ii. developing a guideline for competent authorities on actions that may be taken when residues of veterinary drugs in food are detected at levels below or above Codex Action Levels, or when no Action Levels have been established for adoption.[3]

2. In line with the agreed approach, CCRVDF27 agreed to:

   i. forward the criteria and procedures for the establishment of Action Levels to the 47th session of the Codex Alimentarius Commission (CAC47, 2024) for adoption as Annex D in the Procedural Manual, *Risk Analysis Principles applied by CCRVDF*;

   ii. amend paragraph 133 in the Procedural Manual to include references to Action Levels developed in accordance with Annex D;

   iii. forward the project document for the new work proposal (i.e. to develop a complementary guideline for competent authorities) to CAC47 for approval; and

   iv. include nicarbazin and lasalocid in chicken eggs to the priority list for consideration for Action Levels for adoption as new work by CAC47.[4]

3. CCRVDF27 further agreed to re-establish the Electronic Working Group (EWG), chaired by Canada and co-chaired by Australia and the United States of America (USA), open to all Members and Observers, with the following Terms of Reference (ToRs):

---

1 http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/

2 http://www.fao.org/fao-who-codexalimentarius/committees/committee/related-circular-letters/en/?committee=CCRVDF

3 REP24/RVDF27, para 114

4 REP24/RVDF27, paras 110-113

i. to develop a complementary guideline containing guidance for actions that competent authorities could take upon detection of residues of veterinary drugs in food of animal origin caused by unavoidable and unintentional carryover of veterinary drugs in animal feed pending approval by CAC47; and

ii. to develop Action Levels as approved on the priority list in line with the procedure within Annex D of the *Risk Analysis Principles applied by CCRVDF*.[1]

4. At its 47th session, the Codex Alimentarius Commission (CAC47, 2024)[5]:

i. adopted the criteria and procedures for setting Action Levels proposed by CCRVDF27 and included them in the Procedural Manual, *Risk Analysis Principles applied by CCRVDF*, Annex D.

ii. adopted the amendment to paragraph 133 in the *Risk Analysis Principles applied by CCRVDF*

iii. approved the proposed project document for development of a complementary guideline for competent authorities, as new work for CCRVDF

iv. approved the inclusion of nicarbazin and lasalocid in chicken eggs to the priority list for consideration for Action Levels.

WORK PROCESS: PARTICIPATION AND METHODOLOGY

5. The EWG registered 32 member countries and 2 observer organizations to participate in this work. The List of Participants is presented in Appendix III.

6. The EWG Chairs circulated the first draft document to the EWG members on 16th April 2025 in English. In line with the terms of reference (TOR) of the EWG, the document contained the proposed Action Levels for residues of nicarbazin and lasalocid in chicken eggs, derived according to the procedure within Annex D of the *Risk Analysis Principles applied by CCRVDF*.

7. Two EWG members provided comments on this draft.

8. On the basis of these comments, the EWG Chairs prepared a second draft document and circulated it to the EWG members on the 21st July 2025. One EWG member provided their comments on this draft.

9. The EWG Chairs finalized the EWG's work on Action Levels for residues of nicarbazin and lasalocid in chicken eggs and submitted it to the Codex Secretariat for consideration by Codex members and observers.

SUMMARY OF KEY POINTS OF DISCUSSION

10. One member suggested refinement of the Action Level for nicarbazin by calculating the carryover rate using the highest approved global dose (i.e., 200 mg/kg) and the average carryover rate observed in the study conducted by Martinez et al., 2018. The Chairs agreed that the highest approved dose should be used and noted that Annex D: *Criteria and procedures for the establishment of action levels for residues of veterinary drugs in food of animal origin resulting from unavoidable and unintentional veterinary drug carryover in non-target animal feed*, indicates that the maximum observed concentration of unavoidable and unintended veterinary drug carry-over should be used to derive the carryover rate (Step 1 – Option 2). As such, the Chairs utilized the highest approved dose and maximum observed concentration from the study conducted by Martinez et al., 2018 to revise the Action Level for nicarbazin in chicken eggs from 0.22 mg/kg to 0.35 mg/kg (DNC).

11. This same member also suggested modification of the Action Level for lasalocid by refining the transfer factor derived based on the results of the study conducted by Kennedy et al., 1996. The member noted that the transfer factor had been calculated based on a regression equation reported by the authors, rather than the reported residue values. The Chairs agreed that the reported concentrations should be used to derive the transfer factor. As such, the transfer factor established from the study conducted by Kennedy et al., 1996, was revised from 0.0636 to 0.085. When combined with the transfer factor of 0.118 derived based on the study conducted by Vandenberge et al., 2012, the median transfer factor was determined to be 0.1015. Accordingly, the proposed Action Level for residues of lasalocid in chicken eggs was revised from 0.1 mg/kg to 0.15 mg/kg.

12. The data and information considered by the EWG in the calculation of the action levels are presented in Appendix II. As noted in paragraphs 10 and 11, some of the data in the original calculations required additional refinement within the EWG. Accordingly, the original and refined calculations are highlighted with the original calculations crossed-out.

5 REP24/CAC47, paras 125 and 169, , Appendices II and V

CONCLUSIONS

13. The EWG completed its task in accordance with its ToRs. The outcome is presented in Appendix I, which outlines the proposed action levels for comments by Codex members and observers, and for consideration by CCRVDF28. Appendix II presents the data and information that have been considered by the EWG in applying the procedure described in Annex D of the *Risk analysis principles applied by CCRVDF* to derive the action levels. Specific aspects of the action-level calculations that were refined within the EWG and require CCRVDF's consideration are highlighted in the text and should be considered when providing comments on the proposed action levels.

RECOMMENDATIONS

14. Codex members and observers are invited to consider:

    i. The proposed action levels for residues of nicarbazin and lasalocid in chicken eggs due to the unavoidable and unintentional carryover in feed as presented in Appendix I for comments and consideration by CCRVDF28.

    ii. The scientific assessments, which display the approach used to derive the proposed Action Levels, as presented in Appendix II for information to support comments on the proposed action levels.

APPENDIX I

ACTION LEVELS FOR RESIDUES OF NICARBAZIN AND LASALOCID IN CHICKEN EGGS DUE TO UNAVOIDABLE AND UNINTENTIONAL CARRYOVER IN FEED

(For comments at Step 3)

Table 1: Proposed Action Level for Nicarbazin in Chicken Egg.

Commodity	**Proposed Action Level (mg/kg)**
Egg	0.35
Marker residue - 4,4'-dinitrocarbanilide (DNC)	

Table 2: Proposed Action Level for Lasalocid in Chicken Egg.

Commodity	**Proposed Action Level (mg/kg)**
Egg	0.15
Marker residue - Lasalocid A	

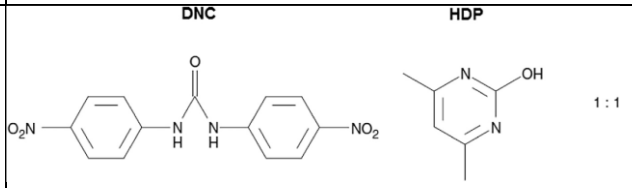
APPENDIX II - Part I

ESTABLISHMENT OF AN ACTION LEVEL FOR RESIDUES OF NICARBAZIN IN CHICKEN EGGS DUE TO UNAVOIDABLE AND UNINTENTIONAL CARRYOVER IN FEED

(For information to support comments on the action levels proposed in Appendix I)

Nicarbazin is a non-ionophoric coccidiostat that is administered in feed to broiler chickens for the prevention and control of coccidiosis caused by *Eimeria* spp. Nicarbazin is an equimolar mixture of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). DNC is also known as N,N'-bis(4-nitrophenyl)urea and 1,3-Bis(4-nitrophenyl)urea. After oral ingestion, the complex dissociates into two major metabolites, DNC and HDP, with both components undergoing metabolism via different routes and at different rates. **Table 1** provides a summary of nicarbazin details.

Table 1: Summary of nicarbazin details

Chemical name	an equimolar mixture of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). DNC is also known as N,N'-bis(4-nitrophenyl)urea.
Marker residue	4,4'-dinitrocarbanilide (DNC)
Structure	 (Tarbin et al., 2005)
Water solubility (20 °C)	DNC - 0.02 mg/L and HDP >10000 mg/L
log K_{ow}	DNC - 3.6 and HDP - 0.94 at pH 5-9 (EFSA, 2003).
Target animal	chickens for fattening, turkeys for fattening
Authorised maximum content in complete feed and Withholding Period (WHP)	125 ppm in the feed, 1 day 40-50 ppm, 0 days (nil) when co-formulated with ionophores (AUS) 100-200 ppm, <125 ppm with 4 days, >125 ppm with 5 days (US) 30-50 ppm with 0 days when co-formulated with narasin (US) 125 ppm, 1 day (EU), 30-50 ppm, 0 days when co-formulated with narasin (EU)
LOQ	0.02 - 0.1 mg/kg for all tissues
ADI	0.9 mg/kg bw (DNC) (JECFA/94/SC, 2022)
MRLs for chicken (broilers) (mg/kg)	***AUS*** muscle 5, liver 35, kidney 20, skin/fat 10, egg 0.3 ***EU*** muscle 4, liver 15, kidney 6, skin/fat 4 ***Canada*** muscle 4, liver 15, kidney 8, skin/fat 4 ***Codex*** muscle 4, liver 15, kidney 8, skin/fat 4 ***US*** liver 52 ***UK VMD*** egg 0.100 (Differential Action Limit, DAL)
Maximum content in feed for non-target species (mg/kg)	***EU Regulation EU 574/2011*** Feed materials- 1.25 Compound feed for equine species, laying birds and chickens reared for laying (> 16 weeks) – 1.25 other animal species – 3.75 ***Brazilian Regulation*** (***MAPA 2016)*** Feed materials - 1.25

Maximum content in food from non-target species (mg/kg)	***EU Regulation (EC) No 124/2009*** Food of animal origin from animal species other than chickens for fattening (mg/kg): Egg 0.3, milk 0.005, liver 0.3, kidney 0.1, other food 0.05 ***New Zealand MRL*** Egg 0.3 ***Australian MRL*** Eggs 0.3

Laying hens are identified as the most likely non-target animals to be exposed to unavoidable and unintentional carry-over of nicarbazin in non-target animal feed, as feed for broiler chickens and laying hens is often prepared at the same feed mill. Survey or residue monitoring data on nicarbazin in poultry eggs **(Table 2)** and feeding studies in laying hens **(Table 4)** provide evidence for the detection of nicarbazin residues in eggs from laying hens fed feed produced in accordance with good manufacturing practices. **Attachment 1** summarizes the residues data for nicarbazin measured in edible tissues from poultry fed nicarbazin containing medicated feed.

A wide range of nicarbazin levels in eggs have been reported by national surveys and residue monitoring programs, with the highest DNC concentration reported at 900 µg/kg **(Table 2)**. As listed in **Table 4**, feeding studies on laying hens resulted in nicarbazin levels in eggs ranging from 226 to 15,300 µg/kg. The variation may be explained in part by the differences in the authorised use-patterns for nicarbazin in broiler chickens. Feeding broiler chickens with a diet containing nicarbazin (in the form of nicarbazin or co-formulated with other ionophores) resulted in residue concentrations ranging from 20-39,770 µg/kg in the liver, 230-5,400 µg/kg in the kidney, 2-6,560 µg/kg in the muscle, and <10-7750 µg/kg in the skin/fat, depending on the different feeding levels, withholding periods (WHPs) and analytical methodologies **(Attachment 1)**. The highest levels of nicarbazin were measured in eggs and poultry liver in comparison to the other edible poultry tissues.

In terms of possible sources of nicarbazin residues in edible commodities of chicken, carry-over of nicarbazin to non-target animal feed during feed manufacturing (Cannavan et al. 2000, Cannavan and Kennedy 2000, McEvoy et al, 2003) has been identified as a source of nicarbazin residues in egg. Several authors have also highlighted that the ingestion of droppings containing excreted (non-absorbed) nicarbazin may pose as possible source of nicarbazin residues in broiler chicken tissues (Cannavan and Kennedy, 2000; Kan et al., 1996).

Table 2: Survey or residue monitoring data on nicarbazin in poultry egg[1]

Country	Year	Commodity	LOQ (mg/kg)	MRL (mg/kg)	No. of samples tested	Positive sample n>LOQ (n>LOD)	Residue levels (µg/kg)	Highest residue level (µg/kg)	Reference
Australia	2011-2021	egg	0.01	0.3	301	13 (28)	<10-66	66	Australian NRS data
Belgium	2005	egg	n/a	n/a	320	13	n/a	10	Mortier et al., 2005
Belgium	a) 2002-03 b) 2005	a) egg b) Poultry, egg, rabbit	a) n/a b) n/a	a) n/a b) n/a	a) n/a b) 6	a) n/a b) n/a	a) 3-197 (4), > 10 (2) b)> 10	a) n/a b) n/a	Mortier et al., 2005
Croatia	2009-2011	poultry egg	0.00015 0.015	0.0005 0.05	a) 307 b) 275	n/a	a) 1.85 b) 21.1	a) 122.8 b) 314.4	Bilandžić et al., 2013
EU	2004 - 2005	egg	0.001-0.1	n/a	3314	23	n/a	n/a	EFSA 2008
Ireland	2002-2004	poultry egg	n/a	n/a	546	9	14-122	122	Danaher et al., 2008
North Ireland	1996-1997	egg (190)	n/a	0.001	190	39	4-342	342	Cannavan and Kennedy 2000
Italy	a) 2012 b) 2013 c) 2014 d) 2016 e) 2017	a) Poultry, ovine, eggs b) Poultry, eggs c) Poultry, eggs d) Poultry, eggs e) Poultry, eggs	0.001 LOQ	n/a	a) 49 (28, 1, 20) b) 49 (31, 18) c) 80 (33, 47) d) 58 (34, 24) e) 46 (34, 12)	a) 4 b) 9 c) 14 d) 20 d) 13	a) 1.4-96 b) 12-21 c) 13-238 d) 13-516 e) 1-321	a) 96 b) 21 c) 238 d) 516 e) 321	Roila et al., 2019
UK	1995-2004	chicken egg	n/a	n/a	2178	123	> 10 DNC	900	UK-VMD, 1995-2004 EFSA 2018
UK	2007	egg	n/a	0.025	234	2	40, 60	60	UK, 2007

[1] Nicarbazin is authorised in the EU and Australia for use in broiler chickens but not approved for laying hens. Residues in the egg are assumed to be from carry-over.

Nicarbazin presence into eggs due to unavoidable and unintentional nicarbazin carry-over in animal feed

Annex D of the Risk Analysis Principles Applied by CCRVDF describes how CCRVDF is to derive action levels for residues of veterinary drugs in foods caused by unavoidable and unintentional carryover of veterinary drugs in animal feed. These procedures are applied to nicarbazin in eggs as described below.

Step 1. Animal dietary exposure assessment

Option 1

A maximum approved dose of 200 mg nicarbazin/kg of complete broiler chicken feed is considered for proposing action levels in eggs from laying hens by utilizing a hypothetical carry-over rate. Carry-over of nicarbazin in laying hen feed at a hypothetical level of 1% of the maximum authorised dose would result in a carry-over level of 2 mg nicarbazin/kg of laying hen feed.

Option 2

Table 3 summarizes the carry-over levels of nicarbazin in non-medicated animal feed following medicated feed manufacturing. The controlled feed mill studies of Martinez et al. (2018) demonstrate that following the manufacturing of medicated feed (containing 125 mg nicarbazin/kg) and subsequent cleaning and flushing procedures (representing good manufacturing practices) carry-over levels of up to 2.2 mg nicarbazin/kg were found in non-medicated feed. This study compared various flushing procedures that reduced the carry-over levels in non-medicated feed. They further claimed that due to nicarbazin's high electrostatic potential, it has a tendency to cling to the bin walls where the product's moisture content and environmental conditions may also play roles in its adhesion.

Table 3: Carry-over levels of nicarbazin in non-medicated animal feed during medicated feed manufacturing

Level in medicated feed (mg/kg)	Flushing procedure	Level in flush (mg/kg)	Level in non-medicated diet (mg/kg)	Reference
125	Five flush size treatments 2.5, 5.0, 10, 15, and 20% of the mixer's total capacity (Forberg 454.5 kg capacity drop bottom paddle mixer)	 19.2 14.8 12.0 6.5 5.6	 1.8 2.1 2.2 1.4 1.5	Martinez et al., 2018
125	Three sequential 3-tonne cleaning batches, sampling before pelleting and at one point post-pelleting		Pre-pelleting (first tonne milled) - 3.4 ± 0.26 Post pelleting (after 8 tonnes) - 7.2± 1.29	McEvoy et al., 2003

Another study (McEvoy et al., 2003) showed that feed batches produced after the intentional incorporation of nicarbazin into feed resulted in carry-over levels as high as 8.49 mg/kg in subsequent batches of feed. A study of German feed-production plants (n≈450) showed carry-over levels of less than 4% in more than half of the examined production plants (W. Strauch, 2002 from EFSA, 2008). Another survey of Belgian compound-feed production companies reported the same level of carry-over in pelleted feeds whereas the mash feeds showed carry-over levels of less than 5% (EFSA, 2008). Studies on carry-over in feed conducted in Italy in 2015 and 2017 reported 0.1-0.8 mg/kg of nicarbazin in non-medicated poultry feed (Roila et al., 2019).

In 2006, the Czech Republic reported 43.5 mg/kg of nicarbazin in one sample of non-medicated pre-mixture for pigs out of 254 samples of different feed commodities (EFSA, 2008). Data for nicarbazin residues from a 2010-2012 Italian survey of non-medicated feedstuff showed a highest carry-over level of 0.46 mg/kg (Moretti et al., 2013), whereas another survey conducted in feedstuffs from feed mills or animal farms in Italy from 2010-2017 showed nicarbazin residues as high as 1.46 mg/kg (Annunziata et al, 2018). Nicarbazin is authorised in the EU and Australia for use in broiler chickens, but not approved for laying hens, so it is assumed residues in egg are due to carry-over.

"The CGMP regulations require medicated feed manufacturers to use one or more of the approved cleanout procedure, such as cleaning, sequencing, and/or flushing to prevent unsafe contamination by drug carryover (Food and Drug Administration, Department of Health and Human Services, 1976). The most effective cleanout procedure is considered the thorough cleaning of the feed manufacturing equipment.

However, given its time-consuming nature and the down time needed to thoroughly clean the equipment, sequencing and flushing are the most commonly used in the feed industry." […] "When it comes to flushing, the FDA recommends using 50–100 g/kg of the mixer's total capacity as the flush material." (Martinez et al., 2018).

Based on the controlled feed mill study of Martinez et al. (2018), under practical conditions (following cleaning and flushing representing GMP), a maximum nicarbazin level of 1.76% (2.2 mg/kg / 125 mg/kg * 100%)~~2.2 mg/kg~~ would be expected in non-medicated feed, due to unavoidable and unintentional carry-over. When applied to the maximum authorised dose of 200 mg/kg, this equates to a carry-over amount of 3.52 mg/kg in non-medicated feed.

Step 2. Estimates of anticipated residue levels in food commodities of animal origin

a) Calculating the transfer factor (TF) for egg

As displayed in **Table 4**, feeding studies in laying hens were used to assess the potential for residues of nicarbazin to transfer from feed to egg. DNC is contained predominantly in egg yolk whereas HDP is found primarily in albumin (Cannavan et al., 2000, Mortier et al., 2005). DNC is the marker residue for nicarbazin. In whole egg, residues were 226 µg/kg upon feeding 1 mg nicarbazin/kg feed (Oishi and Oda, 1989), 7.69 µg/kg at 0.2 mg/kg, 17.96 µg/kg at 0.4 mg/kg, 64.10 µg/kg at 1.3 mg/kg, 192.3 µg/kg at 3.8 mg/kg and 631 µg/kg at 12.1 mg/kg (Cannavan et al., 2000), 300 µg/kg at 2 mg/kg and 6,500 µg/kg at 40 mg/kg (Mortier et al., 2005), 10,000 µg/kg at 200 mg/kg (Nose et al 1982) and 15,300 µg/kg at 147 mg/kg (Johnston et al., 2001).

From **Table 4**, feeding studies with laying hens only fed nicarbazin at levels close to the anticipated carry-over level of 3.52~~2.2~~ mg/kg were used to assess the transfer of nicarbazin from feed to egg (Cannavan et al., 2000 and Mortier et al., 2005). As summarised in **Table 4**, TFs for egg are: 0.051 and 0.150, with the median TF being **0.10** (the Nose et al., 1982 study was not used as issues were observed with animal health and the Oishi and Oda et al., 1989 study was excluded as it is unknown if the nicarbazin values are measured as DNC).

Table 4: Compilation of feeding studies of nicarbazin in laying hens

Species	Feed level (mg/kg)	Duration (days)	LOD (mg/kg)	LOQ (mg/kg)	Residue monitored	Residue level in eggs (µg/kg)	TF_{egg}	Reference
Laying hens*	2 40	14	NS	0.001 CCα 0.012 CCβ	DNC	300 6500	0.150 0.162	Mortier et al., 2005
Laying hens	200	14	NS	NS	DNC	10000	0.05[C]	Nose et al 1982
Laying hens	1.0 0.5 0.1 0.05	10	0.010	NS	DNC	226 - - -	0.226	Oishi and Oda, 1989
Laying hens*	0.2 0.4 1.3 3.8 12.1	16	0.0003	0.001	DNC	7.69 17.96 64.10 192.3 631	0.038[D] 0.045[D] 0.050[D] 0.051[D] 0.052	Cannavan et al., 2000
Laying hens	34.9 54.2 92.5 147	14	0.035[A]	0.117[B]	DNC	4300 9400 13900 15300	0.123 0.173 0.150 0.104	Johnston et al., 2001

*Feeding studies used to calculate TFs.

NS – Not Specified.

[A] LOD = 3 × S/N (Primus et al., 2003)

[B] LOQ = 10 × S/N

[C] laying ceased after 7 days of dosing, restarted after 12 days on non-medicated feed.

[D] TFs were calculated by applying "Y = 0.0195 x + 0.05 equation" derived by Mortier et al., 2005

b) Calculating the anticipated veterinary drug carry-over level in egg

Option 1

Considering carry-over of nicarbazin in laying hen feed at a hypothetical carry-over rate of 1% and assuming a median transfer factor of **0.10**, the expected nicarbazin residue level in egg would be 200 µg/kg (TF_{egg} × carryover level in the feed = 0.10 x 200 mg/kg feed x 1%).

Cannavan et al. (2000) showed a linear relationship between nicarbazin feed intake and levels of DNC in eggs that could be described by the equation presented below. Further, the authors demonstrated that nicarbazin levels in feed above 2 mg/kg results in DNC concentrations in eggs that are greater than the UK differential action limit (DAL) of 100 µg/kg.

Feed-nicarbazin (mg/kg) = 0.0195 × whole egg residue DNC (µg/kg) + 0.05

so

whole egg residue DNC (µg/kg) = (feed nicarbazin (mg/kg) – 0.05)/0.0195

Based on the equation above, DNC residues in whole egg would be estimated to be 100 µg/kg when a hypothetical carry-over rate of 1% of the maximum authorised dose of 200 mg/kg is applied.

Option 2

Based on available feed mill studies, the maximum nicarbazin carry-over rate to non-medicated feed is anticipated to be 1.76%~~2.2 mg/kg~~ (Martinez et al., 2018). When applied to maximum authorised dose of 200 mg/kg, this equates to a carry-over amount of 3.52 mg/kg in non-medicated feed. Utilising this level, the expected nicarbazin residue level in egg would be 352~~220~~ µg/kg (TF_{egg} × carryover level in the feed = 0.10 × 3.52~~2.2~~ mg/kg feed).

Step 3. Action levels

The anticipated nicarbazin residue levels in eggs calculated by using the median TF and a hypothetical carry-over rate of 1% **(Option 1)** or the maximum concentration in feed based on feed mill studies **(Option 2)** are summarized in **Table 5**. The estimated nicarbazin residue level in egg at a 1% hypothetical carry-over level would be 200 µg/kg **(Option 1)**. In contrast, at a carry-over level of 3.52~~2.2~~ mg/kg, the nicarbazin residue level in egg would be estimated at 352~~220~~ µg/kg **(Option 2)**.

Table 5: Summary of the anticipated residue levels in chicken egg

Commodity	**TF**	**Anticipated residue level (µg/kg)**	
		Option 1	**Option 2**
		1% (2 mg/kg feed)	**3.52~~2.2~~ mg/kg feed**
Egg	0.10	200	352~~220~~

The anticipated nicarbazin residue level of 352~~220~~ µg/kg was chosen as the appropriate value to use in the human exposure assessment based on the available feed mill data **(Option 2)** over the conservative default carry-over level of 1%.

Step 4. Human dietary exposure assessment

Noting that JECFA is the appropriate committee to perform **Step 4** (Human dietary exposure assessment), in this assessment, dietary exposure to nicarbazin residues in food resulting from unavoidable and unintentional nicarbazin carry-over in non-target animal feed was assessed using the JECFA TMDI (Theoretical Maximum Daily Intake) as a conservative approach.

The 2022 JECFA established an ADI of 0-900 µg/kg bw (DNC) based on toxicological effects (JECFA/94/SC). Based on the intended use of nicarbazin in broilers considered by the 2022 JECFA, the highest Global Estimates of Chronic Dietary Exposure (GECDE) for infants and toddlers was estimated to be 210 ug/kg bw per day representing 23% of the upper bound of the ADI, based on incurred DNC residues in chicken muscle, offal, and skin with fat, following a 24-hour withdrawal time.

For the expected residue level in eggs, a dietary exposure assessment was performed using the 352~~220~~ µg/kg nicarbazin residue level in eggs, food consumption factor of 100 g of egg and ADI value of 900 µg/kg bw/day **(Table 6).**

As a marker residue to total residue (MR:TR) ratio is not available for eggs, the lowest MR:TR ratio identified by JECFA in the target animal species (kidney – 0.25) has been used to complete the human dietary exposure assessment.

Table 6: Estimation of dietary exposure to nicarbazin (DNC) residues in chicken eggs using JECFA TMDI approach

Commodity	Daily consumption (g)	Anticipated residue level (µg/kg)	MR:TR	TMDI (mg)
Egg	100	352~~220~~	0.25	0.141~~0.088~~
TMDI as %ADI				0.26~~0.16~~%

dietary exposure estimate (TMDI) = 0.141~~0.088~~ mg ÷ 60 kg person/day

= 0.00235~~0.00147~~ mg/kg bw/day

= 0.00235~~0.00147~~ mg/kg bw/day ÷ 0.9 mg/kg bw/day × 100%

= 0.26~~0.16~~% of the ADI

The dietary exposure estimates for nicarbazin residues in egg from non-target animals represents 0.26~~0.16~~% of the ADI. Therefore, it can be considered that there is no appreciable risk to consumers' health from the consumption of egg, produced from laying hens consuming a feed with a carry-over level of 3.52~~2.2~~ mg nicarbazin/kg, regardless of other sources of dietary exposure.

In this assessment, it is proposed to establish an Action Level of 0.35~~0.220~~ mg/kg (rounded down from 0.352 mg/kg) for nicarbazin in eggs from laying hens as non-target animals to accommodate the presence of nicarbazin as a result of unavoidable and unintentional nicarbazin carry-over in animal feed **(Table 7)**. This is in line with similar limits established by EU, New Zealand and Australia for nicarbazin in eggs (0.30~~0.220~~ mg/kg).

Table 7: Proposed action level for nicarbazin in chicken egg

Commodity	Proposed action level (mg/kg)	For comparison - Maximum content (mg/kg)
Egg	0.35~~0.220~~	0.3 (EU) 0.3 (MRL - New Zealand) 0.3 (MRL – Australia)
Marker residue - 4,4'-dinitrocarbanilide (DNC)		

APPENDIX II - Part II Cont.

ESTABLISHMENT OF AN ACTION LEVEL FOR RESIDUES OF LASALOCID IN CHICKEN EGGS DUE TO UNAVOIDABLE AND UNINTENTIONAL CARRYOVER IN FEED

(For information to support comments on the action levels proposed in Appendix I)

Lasalocid sodium (referred to as lasalocid hereafter) is a monocarboxylic polyether ionophore obtained from fermentation of a strain of *Streptomyces*. It is used to control coccidiosis in chickens for fattening, chickens reared for laying, turkeys, and minor avian species (EFSA, 2017). **Table 8** provides a summary of lasalocid details.

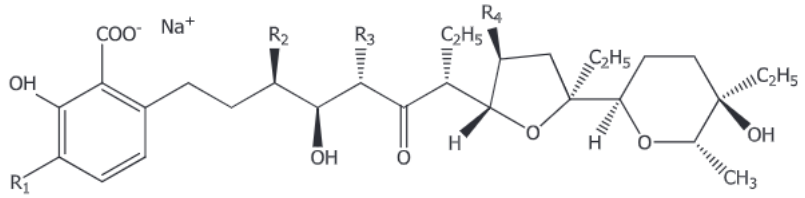
Laying hens are identified as the most likely non-target animals to be exposed to unavoidable and unintentional carry-over of lasalocid in non-target animal feed, as feed for broiler chickens and laying hens is often prepared at the same feed mill. Survey or residue monitoring data on lasalocid in poultry eggs (**Table 9**) and feeding studies in laying hens (**Table 11**) provide evidence for detectable lasalocid levels in eggs from laying hens fed feed produced in accordance with good manufacturing practices. **Attachment 2** summarizes the residue data for lasalocid measured in edible tissues from poultry fed lasalocid containing medicated feed.

Lasalocid is classified as the most potent ionophore in terms of causing unavoidable residues in eggs (Olejnik et al., 2014; Anadon and Martinez-Larranaga, 2014). This is attributed to the fact that eggs are a major route of excretion for lasalocid residues in laying hens. Therefore, even low levels of lasalocid in feed, resulting from unintentional and unavoidable carry-over, can result in the accumulation of residues at reasonably high concentrations in eggs (Wong and Roxburgh, 2010; Kennedy et al., 1996; Vandenberge et al., 2012).

A wide range of lasalocid levels in eggs were reported by national surveys and residue monitoring programs (<1-3450 µg/kg) (**Table 9**). As listed in **Table 11**, feeding studies in laying hens resulted in lasalocid levels in whole egg ranging from 6.36 – 12,000 µg/kg. The variation is likely explained by the concentration of lasalocid incorporated into feed to conduct each study, as lasalocid deposition into egg has been shown to have a nearly linear relationship with the concentration in feed (EFSA, 2007).

In terms of possible sources of lasalocid residues in edible commodities of chicken, carry-over of lasalocid to non-target animal feed during feed manufacturing has been identified as a source of lasalocid residues in egg (Kennedy et al., 1996; Vandenberge et al., 2012). Even if all preventative measures are followed, such as the use of rinsing batching, cross-contamination of residues is unavoidable under practical conditions (EFSA, 2007). Additionally, lasalocid is a very dusty compound, which can easily contaminate feed during the manufacturing process (Rokka et al., 2013).

Table 8: Summary of lasalocid details

Chemical Name	Lasalocid sodium
Marker Residue	Lasalocid A
Structure	 (EFSA, 2017)
Water Solubility (20 ℃)	1,060 mg/L (EFSA, 2017)
log K_{ow}	2.3 (EFSA, 2017)
Target Animal	Chickens for fattening, chickens reared for laying, turkeys for fattening
Authorized maximum content in complete feed and Withholding Period (WHP)	100-105 mg/kg (broiler chickens and turkeys; Canada) – zero-day (meat) 75-125 mg/kg (broiler chickens and turkeys; US) – zero-day (meat) 75-125 mg/kg (turkeys; UK) – 5-day (meat) 75-100 mg/kg (broiler chickens and replacement pullets; Australia) – 3-day (meat) and 14-day (egg) 90-125 mg/g (turkeys; Australia) – zero-day (meat) 75-100 mg/kg (broiler chickens and replacement pullets; New Zealand) – zero-day (meat) 14-day (egg) 90-125 mg/g (turkeys; New Zealand) – zero-day (meat)

LOQ	5 µg/kg for all tissues
ADI	0-5 µg/kg bw/day (JECFA/78/SC, 2013)
MRLs for chicken (broilers/layers) (mg/kg)	***AUS*** skin/fat 0.6, kidney 0.7, liver 1.2, muscle 0.4, eggs 0.05 ***Canada*** kidney 0.4, liver 0.4, muscle 0.1, skin/fat 0.4 ***Codex*** muscle 0.4, liver 1.2, kidney 0.6, skin/fat 0.6 ***EU*** muscle 0.06, skin/fat 0.3, liver 0.3, kidney 0.15, eggs 0.15 ***UK VMD*** muscle 0.06, liver 0.3, kidney 0.15, skin/fat 0.3, eggs 0.1 ***US*** skin/fat 1.2, liver 0.4
Maximum content in feed for non-target species (mg/kg)	***EU Regulation EU 574/2011*** Feed materials - 1.25 Compound feed for dogs, calves, rabbits, equine species, dairy animals, laying birds, turkeys (> 16 weeks) and chickens reared for laying (> 16 weeks) – 1.25 Compound feed for chickens for fattening, chickens reared for laying (< 16 weeks) and turkeys (< 16 weeks) for the period before slaughter in which the use of lasalocid sodium is prohibited (withdrawal feed) – 1.25 Other animal species – 3.75
Maximum content in food from non-target species (mg/kg)	***EU Regulation EU 610/2012*** Food of animal origin from animal species other than poultry and bovine: Milk – 0.001 Liver – 0.05 Kidney – 0.02 Other food – 0.005

Table 9: Survey or residue monitoring on lasalocid in poultry egg

Country	Year	Commodity	LOQ (mg/kg)	MRL (mg/kg)	No. of samples tested	Positive sample n>LOQ (n>LOD)	Residue levels (µg/kg)	Highest residue level (µg/kg)	Reference
Australia	2021-22	Whole egg	Limit of Reporting (LOR) – 0.01	0.05	30	1	>LOR to ≤½MRL	NS	Australian NRS, 2021-2022
EU	2005	Egg	CCα = 0.001	MRL NS AL – 0.01 (Belgian Food Agency)	320	24	<1-49	49	Mortier et al., 2005
(Northern) Ireland	1994	Egg	0.001	-	161	107	0.3-129	129	Kennedy et al., 1996; Kennedy et al., 1998
(Northern) Ireland	1995	Egg	0.001	-	220	45 (= 220*0.205)	<1 to >10	NS	Kennedy et al., 1998
Belgium	2004	Egg	-	-	190	-	4-90	90	Mortier et al., 2005
UK	1995-2005	Egg	-	0.01	2855	138	<2-3,450	3450	EFSA, 2007
Italy	2012 2013 2014 2015 2016 2017	Egg	0.001	-	353 animal tissue and egg samples	2 - 1 4 (2) 1 -	1.5-2.0 - 1.2 2.8-1,002 26 -	1,002	Roila et al., 2019

Country	Year	Commodity	LOQ (mg/kg)	MRL (mg/kg)	No. of samples tested	Positive sample n>LOQ (n>LOD)	Residue levels (µg/kg)	Highest residue level (µg/kg)	Reference
UK	1998	Egg	-	0.002[a]	221	5	<1-3500	3500	Wong and Roxburgh, 2010
	1999			0.002	208	21			
	2000			0.002	212	20			
	2001			0.002	222	12			
	2002			0.04	280	18			
	2003			0.05	275	33			
	2004			0.05	283	8			
	2005			0.05	294	4			
	2006			0.05	249	4			
	2007			0.15	218	0			

NS – Not Specified.

AL – Action Limit.

[a] Defined as action level/maximum residue limit for each specific year.

Lasalocid presence in eggs due to unavoidable and unintentional lasalocid carry-over in animal feed

Annex D of the Risk Analysis Principles Applied by CCRVDF describes how CCRVDF is to derive action levels for residues of veterinary drugs in foods caused by unavoidable and unintentional carryover of veterinary drugs in animal feed. These procedures are applied to lasalocid in eggs as described below.

Step 1. Animal dietary exposure assessment

Option 1

A maximum approved dose of 125 mg lasalocid/kg of complete broiler chicken feed is considered for proposing action levels in eggs from laying hens by utilizing a hypothetical carry-over rate. Carry-over of lasalocid in laying hen feed at a hypothetical level of 1% of the maximum authorised dose would result in a carry-over level of 1.25 mg lasalocid/kg of laying hen feed.

Option 2

Table 10 summarizes the carry-over levels of lasalocid in non-medicated animal feed following medicated feed manufacturing, as reported by Kennedy et al. (1996, 1998). In one study, (Kennedy et al., 1996) a feed mill prepared a four-ton batch of medicated turkey grower meal using a powdered lasalocid drug premix to provide a final therapeutic concentration of 100 mg lasalocid/kg of feed. The level of lasalocid carry-over was assessed in nine subsequent batches of non-medicated feed. The first batch of non-medicated feed contained 6 mg lasalocid/kg of feed, while the ninth batch of non-medicated feed contained lasalocid concentrations ranging from 0.5-1 mg/kg. In an analogous experiment, cross-contamination of lasalocid from a medicated premix to successive batches of non-medicated premix was measured, with only the first batch of non-medicated premix containing appreciable levels of lasalocid (level not reported) (Kennedy et al., 1996).

In a second study conducted by Kennedy et al. (1998), carry-over of lasalocid into non-medicated feed was evaluated following the manufacturing of medicated feed (100 mg lasalocid/kg of feed) using a granular lasalocid premix. Lasalocid remained present in the first four batches of non-medicated feed but could not be detected in the subsequent five batches. When compared to the results of the study conducted by Kennedy et al. in 1996, the extent of carry-over into non-medicated feed was significantly reduced when medicated feed was manufactured using the granular premix.

In both studies described above, there was no indication of any mitigation steps representing good manufacturing practices being performed.

Table 10: Carry-over levels of lasalocid in non-medicated animal feed during medicated feed manufacturing

Level in medicated feed (mg/kg)	Flushing procedure	Level in flush (mg/kg)	Level in non-medicated diet (mg/kg)	Reference
100	-	-	Batch one – 6 Batch nine – 0.5-1	Kennedy et al., 1996
100	-	-	Batch 1 – 3.2 Batch 4 – 0.25	Kennedy et al., 1998

A study of German feed-production plants (n≈450) showed carry-over levels of less than 4% in more than half of the examined production plants (W. Strauch, 2002 from EFSA, 2007). Another survey of Belgian compound-feed production companies reported the same level of carry-over in pelleted feeds whereas mash feeds showed carry-over level of less than 5% (EFSA, 2007). Studies on carry-over in feed conducted in Italy from 2012 to 2017 reported lasalocid levels in non-medicated poultry feed between 0.1-5.9 mg/kg (Roila et al., 2019). A Swiss feed plant that produces feed for chickens for fattening and laying hen meal found that one production passage without the addition of lasalocid was not sufficient to reduce the contents of the drug in laying hen meal below 30 µg/kg (Noser et al., 2006).

In 2007, following a request from the European Commission, the Panel on Contaminants in the Food Chain was asked to deliver a scientific opinion on cross-contamination of non-target feedingstuffs by lasalocid authorized for use as a feed additive. The Czech Republic analyzed 254 samples from feed materials for non-target animal species in 2006. One positive sample, a complete feeding stuff for pigs, contained 8.41 mg lasalocid/kg feed (EFSA, 2007).

Denmark reported the analyses of 111 feed samples for non-target species that were collected between 2004 and 2007 and found one positive sample that contained 0.26 mg lasalocid/kg feed (EFSA, 2007). Information from the Rapid Alert System for Food and Feed (RASFF) between April 2002 and April 2006 showed nine incidences where feed for non-target animal species contained lasalocid. The amounts detected were between 0.003 and 12.07 mg lasalocid/kg feed, with one outlier containing 64.6 mg/kg feed. The outlier is most likely due to accidental contamination (EFSA, 2007).

Based on the feed mill study of Kennedy et al., 1998, a maximum lasalocid level of 3.2 mg/kg may be expected in non-medicated feed. However, it is unclear whether mitigation steps representing GMP were performed during this study. Indeed, "the CGMP regulations require medicated feed manufacturers to use one or more of the approved cleanout procedure, such as cleaning, sequencing, and/or flushing to prevent unsafe contamination by drug carryover (Food and Drug Administration, Department of Health and Human Services, 1976). The most effective cleanout procedure is considered the thorough cleaning of the feed manufacturing equipment. However, given its time-consuming nature and the down time needed to thoroughly clean the equipment, sequencing and flushing are the most commonly used in the feed industry." […] "When it comes to flushing, the FDA recommends using 50–100 g/kg of the mixer's total capacity as the flush material." (Martinez et al., 2018). Consequently, the anticipated exposure level for non-target animals from feed could not be estimated.

Step 2. Estimates of anticipated residue levels in food of animal origin

a. Calculating the transfer factor (TF) for egg

As displayed in **Table 11**, feeding studies in laying hens were used to assess the potential for residues of lasalocid to transfer from feed to egg. Lasalocid A is the marker residue for lasalocid. ~~For the study performed by Kennedy et al. (1996), residues in whole eggs were calculated using the following equation:~~

~~Concentration in eggs (ug/kg) = 63.6 x Concentration in feed (mg of lasalocid/kg of feed)~~

In whole egg, residue concentrations were 10.0~~6.36~~ µg/kg upon feeding 0.1 mg lasalocid/kg feed, 30.0~~19.08~~ µg/kg at 0.3 mg/kg, 35.0~~1.8~~ µg/kg at 0.5 mg/kg, 85.0~~63.6~~ µg/kg at 1.0 mg/kg, and 300.0~~18~~ µg/kg at 5.0 mg/kg (Kennedy et al., 1996), 370 µg/kg at 3.125 mg/kg, 780 µg/kg at 6.25 mg/kg, and 1,410 µg/kg at 12.5 mg/kg (Vandenberge et al., 2012), 11,000-12,000 µg/kg at 125 mg/kg (EMA, 2006) and 2.0 µg/kg at 1 mg/kg (Rokka et al., 2005).

As suitable data is not available to establish, with certainty, that unintended and unavoidable carry-over of lasalocid would occur at a level higher than 1% of the maximum authorized dose (125 mg/kg), a default hypothetical carry-over rate of 1% (1.25 mg/kg) was considered. From **Table 11,** feeding studies in laying hens only fed lasalocid at levels close to the carry-over level of 1.25 mg/kg were used to assess the potential for lasalocid transfer from feed to egg (Kennedy et al., 1996 and Vandenberge et al., 2012).

As summarized in **Table 11,** TFs for egg are 0.085~~0.0636~~ and 0.118, resulting in a median TF of **0.1015~~0.0908~~**. The TF determined by Rokka et al. (2005) was excluded as it did not state the concentration of lasalocid in whole egg, but only in egg yolk. The study from the EMA (2006) was excluded as the laying hens were fed lasalocid at a therapeutic concentration, therefore the TF may be overestimated.

Table 11: Compilation of feeding studies of lasalocid on laying poultry

Species	Feed level (mg/kg)	Duration (days)	LOD (µg/kg)	LOQ (µg/kg)	Residue monitored	Residue levels in eggs (µg/kg)	TF_{egg}	Reference
Laying hens*,[a]	0.1 0.3 0.5 1.0 5.0	16	0.3	1.0	Lasalocid A	~~6.36~~10.0 (d6) ~~19.08~~ 30.0 (d10) ~~31.8~~ 35.0 (d8) ~~63.6~~ 85.0 (d12) ~~318~~ 300.0 (d16)	~~0.0636~~ 0.1 0.1 0.07 0.085 0.06	Kennedy et al., 1996

Species	Feed level (mg/kg)	Duration (days)	LOD (µg/kg)	LOQ (µg/kg)	Residue monitored	Residue levels in eggs (µg/kg)	TF_{egg}	Reference
Laying hens*,[a]	3.125 6.25 12.5	14d + 17d depletion period	-	2	Lasalocid A	370 (d16) 780 (d11) 1410 (d14)	0.118 0.125 0.113	Vandenberge et al., 2012
Laying hens	125	12	0.093	10	Total Radioactive Residues	11000-12000 (whole egg; total residues) 291 (egg white) 32500 (egg yolk)	0.088-0.096	EMA, 2006
Laying hens	1.0	21	CCB – 2.0	-	Lasalocid A	2.0 (egg yolk)	0.002	Rokka et al., 2005
* The peak residue concentration was used to determine the residue levels in eggs. [a] Feeding studies used to calculate TFs. NS – Not Specified.								

b. Calculating the anticipated veterinary drug carry-over level in egg

Option 1

Considering carry-over of lasalocid in laying hen feed at a hypothetical carry-over rate of 1% and assuming a median transfer factor of 0.1015~~0.0908~~, the expected lasalocid residue level in egg would be 126.9~~113.5~~ µg/kg (TF_{egg} x carryover level in the feed = 0.1015~~0.0908~~ x 125 mg/kg feed x 1%).

Option 2

The anticipated exposure level for non-target animals could not be estimated based on the feed mill study of Kennedy et al. (1998), as GMP could not be verified.

Step 3. Action levels

The anticipated lasalocid residue level in egg calculated by using the median TF and hypothetical carry-over rate of 1% **(Option 1)** is summarized in **Table 12**.

Table 12: Summary of the anticipated residue levels in chicken egg

Commodity	TF	Anticipated residue level (µg/kg)	
		Option 1 1% (1.25 mg/kg feed)	Option 2 -
Egg	0.1015~~0.0908~~	126.9~~113.5~~	-

The anticipated lasalocid residue level of 126.9~~113.5~~ µg/kg, based on a default hypothetical carry-over rate of 1%, was chosen as the appropriate value to use in the human exposure assessment (**Option 1**). A default carry-over level of 1% was considered as there are no data demonstrating the amount of unavoidable and unintentional veterinary drug carry-over in feed occurring after mitigation steps have been performed.

Step 4. Human dietary exposure assessment

Noting that JECFA is the appropriate committee to perform **Step 4** (Human Dietary Exposure Assessment), in this assessment, dietary exposure to lasalocid residues in food resulting from unavoidable and unintentional lasalocid carry-over in non-target animal feed was assessed using the JECFA TMDI (Theoretical Maximum Daily Intake) as a conservative approach.

The 2013 JECFA established an ADI of 0-5 µg/kg bw for lasalocid based on a NOAEL of 0.5 mg/kg body weight per day derived from a toxicity study in rabbits (JECFA/78/SC, 2013). The estimated daily intake (EDI) was calculated as 80 µg/person/day, based on median residues for a 1-day withdrawal in chicken, which represents approximately 27% of the upper bound of the ADI, based on a 60 kg individual (JECFA/78/SC, 2013). The Global Estimates of Chronic Dietary Exposure (GECDE) for the general population was 1.85 ug/kg bw per day, which represents 37% of the upper bound of the ADI. The GECDE for children was 3.38 ug/kg per day, which represents 67% of the upper bound of the ADI. The GECDE for infants was 2.99 ug/kg bw per day, which represents 60% of the upper bound of the ADI (FAO/WHO, 2015).

For the expected carry-over of residues in eggs, a dietary exposure assessment was performed using the 126.9~~113.5~~ µg/kg lasalocid A residue level in eggs, food consumption factor of 100 g of egg, marker residue to total residue (MR:TR) ratio of 0.38 (EMA, 2006) and ADI value of 5 µg/kg bw/day **(Table 13)**.

Table 13: Estimation of dietary exposure to total lasalocid residues in chicken eggs using JECFA TMDI approach

Commodity	Daily consumption (g)	Anticipated residue level (µg/kg)	MR:TR	TMDI (mg)
Egg	100	126.9~~113.5~~	0.38	0.033~~0.03~~
TMDI as % ADI				11~~10~~ %

Dietary exposure estimate (TMDI) = 0.033~~0.03~~ mg ÷ 60 kg person/day

= 0.0005 mg/kg bw/day

= 0.00055~~0.0005~~ mg/kg bw/day ÷ 0.005 mg/kg bw/day x 100%

= 11~~10~~ % of the ADI

The dietary exposure estimates for lasalocid residues in eggs from non-target animals represent 11~~10~~% of the ADI. Therefore, it can be considered that there is no appreciable risk to consumers' health from the consumption of eggs produced from laying hens consuming a feed with a carry-over level of 1.25 mg lasalocid/kg, regardless of other sources of dietary exposure.

In this assessment, it is proposed to establish an Action Level of 0.15~~0.01~~ mg/kg (rounded up~~down~~ from 0.1269~~0.113~~ mg/kg) for lasalocid in eggs from laying hens as non-target animals to accommodate the presence of lasalocid as a result of unavoidable and unintentional lasalocid carry-over in animal feed **(Table 14)**.

Table 14: Proposed action level for lasalocid in chicken egg

Commodity	Proposed action level (mg/kg)	[For comparison] Maximum content (mg/kg)
Egg	0.15~~0.1~~	0.15 (MRL - EU) 0.05 (MRL - Australia)
Marker residue – Lasalocid A		

References

1. ANADA 200-027. Abbreviated New Animal Drug Applications (ANADAs). FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. NICARMIX (nicarbazin) 25%. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/960
2. Annunziata L., Visciano P., Stramenga A., Colagrande M. N., Campana G., Scortichini G., Migliorati G., Compagnone D. 2018. Investigation of Nonionophoric Coccidiostat Residues in Feed as a Consequence of Carryover. *J Food Prot*, 23:482-489. https://doi.org/10.4315/0362-028X.JFP-17-414
3. Bilandžić N., Dolenc J., Gačnik K.Š., Varenina I., Kolanović B.S. 2013. Feed additives diclazuril and nicarbazin in egg and liver samples from Croatian farms. *Food Addit Contam Part B Surveill,* 6(2):90-7. https://doi.org/10.1080/19393210.2012.727474
4. Cannavan A., Kennedy D.G., 2000. Possible causes of nicarbazin residues in chicken tissues. *Food Addit Contam,* 17:1001–1006 https://doi.org/10.1080/0265203001001325
5. Cannavan A., Ball G., Kennedy D.G. 2000. Nicarbazin contamination in feeds as a cause of residues in eggs. *Food Addit Contam*, 17:829-836. https://doi.org/10.1080/026520300420394
6. Codex Alimentarius Code of Practice on Good Animal Feeding (CAC/RCP 54-2004) https://www.fao.org/fao-who-codexalimentarius/thematic-areas/animal-feed
7. Codex Alimentarius Guidelines on the Application of Risk Assessment for Feed (CAC/GL 80-2013). Codex Alimentarius Commission. https://www.fao.org/feed-safety/resources/resources-details/en/c/1054051/
8. Danaher M., Campbell K., O'Keeffe M., Capurro E., Kennedy G., Elliott C.T. 2008. Survey of the anticoccidial feed additive nicarbazin (as dinitrocarbanilide residues) in poultry and eggs. *Food Addit Contam*. 25:32-40. https://doi.org/10.1080/02652030701552956
9. EFSA (European Food Safety Authority), 2003. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request from the Commission on the efficacy and safety of the coccidiostat Koffogran. The EFSA Journal, 16, 1-40. https://doi.org/10.2903/j.efsa.2004.16
10. EFSA (European Food Safety Authority), 2008. Cross-contamination of non-target feedingstuffs by nicarbazin authorised for use as a feed additive. Scientific opinion of the Panel on Contaminants in the Food Chain. EFSA J. 690, 1–34. https://doi.org/10.2903/j.efsa.2008.690
11. EFSA (European Food Safety Authority), 2010a. Scientific Opinion on the safety and efficacy of Koffogran (nicarbazin) as a feed additive for chickens for fattening. EFSA Journal, 8(3):1551. https://doi.org/10.2903/j.efsa.2010.1551
12. EFSA (European Food Safety Authority), 2010b. Scientific opinion on the safety and efficacy of Maxiban G160 (narasin and nicarbazin) for chickens for fatting. EFSA Panel on Additives and Products or Substances used in Animal Feed). EFSA J. 8 (4), 1754. https://doi.org/10.2903/j.efsa.2010.1574
13. EFSA (European Food Safety Authority), 2017. Safety and efficacy of Monimax® (monensin sodium and nicarbazin) for turkeys for fattening. EFSA Journal 2017;15(12):5094. https://doi.org/10.2903/j.efsa.2017.5094
14. EFSA (European Food Safety Authority), 2018a. Safety and efficacy of Coxar® (nicarbazin) for turkeys for fattening. EFSA Journal 2018;16(4):5214.
15. EFSA (European Food Safety Authority), 2018b. Safety and efficacy of Monimax® (monensin sodium and nicarbazin) for chickens for fattening and chickens reared for laying. EFSA Journal 2018;16(11):5459. https://doi.org/10.2903/j.efsa.2018.5214
16. EFSA (European Food Safety Authority), 2021. Safety and efficacy of a feed additive consisting of lasalocid A sodium and nicarbazin (NilablendTM 200G) for chickens for fattening (Zoetis Belgium SA). EFSA Journal 2021;19(3):6466. https://doi.org/10.2903/j.efsa.2021.6466
17. FAO/WHO (Food and Agriculture Organisation/World Health Organization), 1999. Evaluation of certain veterinary drug residues in food. Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 888. http://whqlibdoc.who.int/trs/WHO_TRS_888.pdf.
18. FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES Ninety-fourth meeting (Residues of veterinary drugs) Virtual meeting, 16–27 May 2022 (JECFA/94/SC)
19. Johnston J. J., Britton W.M., MacDonald A., Primus T. M., Goodal M. J., Yoder C. A., Miller L. A., Fagerstone K. A. 2001. Quantification of plasma and egg 4, 4'-dinitrocarbanalide (DNC) residues for the efficient development of a nicarbazin based contraceptive for pest waterfowl. Pest Manage Sci, 58, 197–202.

20. Kan C. A., Keukens H. J., Boers E. (1995) Nicarbazin residues in broiler muscle, liver and litter/faeces: early exposure or recirculation. 12th European Symposium on the Quality of Poultry Meat 1: 481-485.
21. Konieczka P., 2.31 - Validation and Regulatory Issues for Sample Preparation, Editor(s): Janusz Pawliszyn, Comprehensive Sampling and Sample Preparation, Academic Press, 2012, Pages 699-711, ISBN 9780123813749, https://doi.org/10.1016/B978-0-12-381373-2.00064-8.
22. Kramer H. T. 1990. Determination of Nicarbazin in Chicken Tissues by High Performance Liquid Chromatography. Report by Hazelton Laboratoorires for Planalquimica Industrial, LTDA Sao Paulo, Brazil.
23. Martinez A., Jones C. K., Stark C. R., McKinney L. J., Behnke K. C., Paulk C. B. 2018. Evaluating flushing procedures to prevent nicarbazin carryover during medicated feed manufacturing, Animal Feed Science and Technology, 242:1-7, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2018.04.017.
24. Martinez-Kawas A. 2008. Evaluating Flushing Procedures to Prevent Drug Carryover during Medicated Feed Manufacturing, Master Science Thesis, Universidad Autonoma de Nuevo Leon,
25. McEvoy J.D., Smyth W.G., Kennedy D.G. 2003. Contamination of animal feedingstuff with nicarbazin: investigations in a feed mill. *Food Addit Contam*. 20: 136-40. https://doi.org/10.1080/0265203021000050608
26. MAPA 2016a. Ministério da Agricultura, Pecuária e Abastecimento. 2016. Instrução Normativa Nº 14: anexo II: critérios para manipulação de medicamento veterinário em fábricas de produtos destinados à alimentação animal. [Ministry of Agriculture, Livestock and Supply (MAPA). Normative Instruction No. 14: annex II: criteria for handling of veterinary medicinal products in factories of products intended for animal feed]. [accessed 2016 Jul 08]. http://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/21288772/do1-2016-07-08-instrucao-normativa-n-14-de-6-de-julho-de-2016-21288711.
27. MAPA 2016b. Ministério da agricultura pecuária e abastecimento. 2016b. Instrução Normativa Nº 14: anexo II: critérios para manipulação de medicamento veterinário em fábricas de produtos destinados à alimentação animal. Brasília, 15 de julho de 2016 [Normative Instruction No. 14: Annex II: criteria for handling of veterinary medicine in factories of products intended for animal feed. Brasília, July 15, 2016].
28. Mortier L., Huet A. C., Charlier C., Daeselaire E., Delahaut P., Van Peteghem , C. 2005a. Incidence of residues of nine anticoccidials in eggs. *Food Addit Contam* 22: 1120-1125. https://doi.org/10.1080/02652030500199355
29. Mortier L., Huet A. C., Daeseleire E., Huyghebaert G., Fodey T., Elliott C., Delehaut P. and Van Peteghem C. 2005b. Deposition and depletion of five anticoccidials in eggs. *J Agric Food Chem*. 53: 7142-7149. https://doi.org/10.1021/jf050836r
30. Moretti S., Fioroni L., Giusepponi D., Pettinacci L., Saluti G., Galarini R. 2013. Development and validation of a multiresidue liquid chromatography/tandem mass spectrometry method for 11 coccidiostats in feed. *J AOAC Int*, 96:1245-1257. https://doi.org/10.5740/jaoacint.12-440
31. NADA 138-952a. New Animal Drug Application (NADA). FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. 1989. MAXIBAN® Narasin and Nicarbazin Type A Article. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/439
32. NADA 138-952b. New Animal Drug Application (NADA). FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. 2018. Maxiban™ 72 narasin and nicarbazin Type A medicated article Broiler chickens. Residue Depletion of Nicarbazin and Narasin in Edible Tissues from Chickens Following Administration of Maxiban®2G160 via Feed (Study No. T4HAUK0703). https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/3875
33. NADA 141-124. New Animal Drug Application (NADA). FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary, 2002. MAXIBAN® + BMD®(narasin and nicarbazin + bacitracin methylene disalicylate). https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/650
34. NADA 141-113. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary.Combined use of MAXIBAN® and 3-NITRO® in Chicken Feeds. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/644

    Note: This product is no longer approved for use in the United States.
35. NADA 141-112. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. Combined use of MAXIBAN®, BMD®, and 3-NITRO® in Broiler Chicken Feeds. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/643

    Note: This product is no longer approved for use in the United States.

36. NADA 140-926. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. Combined use of MAXIBAN® and BMD® in Chicken Feeds. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/489

37. NADA 140-339. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. Nicarbazin (NICARB®) plus Bambermycins (FLAVOMYCIN®) https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/478

38. NADA 140-947. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary, 1998. Maxiban®, Lincomix®. Experiment No. AAC8814. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/2501

    Note: This product is no longer approved for use in the United States.

39. NADA 141-529. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary, 2020. Pennitracin MD® and Maxiban™. Study No. AAC8721. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/9748

40. Nose N., Hoshino Y., Kikuchi Y., Masaki H., Horie S., Kawauchi S.(1982) Residues of synthetic antibacterial feed additives in tissues and eggs of chickens. Shokuhin Eiseigaku Zasshi, 23, 246–252O'Keeffe M., Capurro E., Danaher M., Campbell K., Elliott C. T. 2007. Investigation of the causes for the occurrence of residues of the anticoccidial feed additive nicarbazin in commercial poultry, Food Addit Contam, 24:9, 923-934, https://doi.org/10.1080/02652030701258778

41. Oishi Y., Oda T. 1989. Studies on residues of nicarbazin in eggs of laying hens. Shokuhin Eiseigaku Zasshi. Food Hygiene and Safety Science) 30: 542-547.

42. Penz A. M. Jr., Vieira S. L., Ludke J. V. 1999, Nicarbazin residues in broiler tissue and litter. J Poult Sci, 8, 292-297 https://doi.org/10.1093/japr/8.3.292

43. Pietruk K., Olejnik M., Jedziniak P., Szprengier-Juszkiewicz T. 2015. Determination of fifteen coccidiostats in feed at carry-over levels using liquid chromatography-mass spectrometry. *J Pharm Biomed Anal,* 112:50-59. https://doi.org/10.1016/j.jpba.2015.03.019

44. van Rhijn H., Yedema E., Beek W., Kan C. A. (Kees) & Sebek, Leon & Keukens, H. (2000). Investigation into the excretion of nicarbazin in cows milk after administration of feeds containing low-levels of nicarbazin. Proceedings of the EuroResidue IV Conference, Veldhoven, The Netherlands.

45. Roila R., Branciari R., Pecorelli I., Cristofani E., Carloni C., Ranucci D., Fioroni L. 2019. Occurrence and Residue Concentration of Coccidiostats in Feed and Food of Animal Origin; Human Exposure Assessment. *Foods,* 8(10):477 https://doi.org/10.3390/foods8100477

46. Strauch W. 2002. Causes and control of carry-over and cross-contamination. Kraftfutter/Feed Magazine 85 (4): 151-159 (Part 1). Kraftfutter/Feed Magazine 85 (6): 239-249 (Part 2).

47. Tarbin J. A., Bygrave J., Bigwood T., Hardy D., Rose M., Sharman M. 2005. The effect of cooking on veterinary drug residues in food: Nicarbazin (Dinitrocarbanilide component). Food Addit Contam, 22(11), 1126–1131. http://dx.doi.org/10.1080/02652030500357193.

48. UK-VMD (United Kingdom-Veterinary Medicines Directorate), 1995-2004. The Veterinary Medicines Directorate Annual Reports on Surveillance for Veterinary Residues for 1995-2004. Published by the Veterinary Medicines Directorate, New Haw, Surrey, England. http://www.vmd.gov.uk/Publications/AnnReps/AnnReps.htm

49. UK 2007 Annual report on surveillance for veterinary residues in food in the UK 2007 https://www.gov.uk/government/publications/annual-report-on-surveillance-for-veterinary-residues-in-food-in-the-uk-2007

50. Wood J. S., Downing G. G. 1980. Modified Pulse Polarographic Determination of Nicarbazin in Chicken Tissue at the 0.1 Part-per-Million Level, *J. Agric and Food Chem,* 28:452-454

51. Anadon, A., Martinez-Larranaga, M.R. 2014. Veterinary drugs residues: coccidiostats. Encyclopedia of Food Safety, 3:63-75.

52. Australia New Zealand Food Standards Code – Schedule 20 – Maximum residue limits. Australia New Zealand Food Standards Code – Schedule 20 – Maximum residue limits (legislation.gov.au)

53. Bello, A., Henri, J., Viel, A., Mochel, J. P., Pozniak, B. 2023. Ionophore coccidiostats – disposition kinetics in laing hens and residues transfer to eggs. Poultry Science, 102(1):102280.

54. Codex Alimentarius Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Foods (CX/MRL 2-2021). MAXIMUM RESIDUE LIMITS (MRLs) AND RISK MANAGEMENT RECOMMENDATIONS (RMRs) (fao.org)

55. Codex Alimentarius Veterinary Drug Details Lasalocid Sodium. Veterinary Drug Detail | CODEXALIMENTARIUS FAO-WHO

56. Dorne, J.L.C.M., Fernandez-Cruz, M.L., Bertelsen, U., Renshaw, D.W., Peltonen, K., Anadon, A., Feil, A., Sanders, P., Wester, P., Fink-Gremmels, J. 2013. Risk assessment of coccidiostatics during feed cross-contamination: animal and human health aspects. Toxicology and Applied Pharmacology, 270(3):196-208.

57. EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the reevaluation of coccidiostat Avatec in accordance with article 9G of Council Directive 70/524/EEC.

58. EFSA (European Food Safety Authority). 2007. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on Cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive. Cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive - Scientific Opinion of the Panel on Contaminants in the food Chain (wiley.com)

59. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M.L., Bories, G., Chesson, A., Cocconcelli, P.S., Flachowsky, G., Kolar, B., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R.E., Wallace, R.J., Wester, P., Brantom, P., Halle, I., van Beelen, P., Holczknecht, O., Vettori, M.V., Gropp, J. 2017. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying, and modification of the terms of authorisation for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. EFSA J. 2017; 15(8):4857, 32 pp. https://doi.org/10.2903/j.efsa.2017.4857

60. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M.L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R.E., Woutersen, R., Gropp, J., Rychen, G., Anguita, M., Holczknecht, O., Innocenti, M.L., Navarro-Villa, A., Rossi, B., Vettori, M.V. 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of lasalocid A sodium (Avatec® 150G) for chickens for fattening and chickens reared for laying (Zoetis Belgium SA). EFSA J. 2022; 20(12):7715, 14 pp. https://doi.org/10.2903/j.efsa.2022.7715

61. EMA (European Medicines Agency), 2004. Lasalocid Sodium. Summary Report of the Committee for Veterinary Medicinal Products. EMEA/MRL/912/04-FINAL, October 2004. https://www.ema.europa.eu/en/documents/mrl-report/lasalocid-sodium-summary-report-committee-veterinary-medicinal-products_en.pdf

62. EMA (European Medicines Agency), 2006. Lasalocid Sodium (extension to eggs). Summary Report of the Committee for Medicinal Products for Veterinary Use. EMEA/CVMP/46049/2006-FINAL-corr, July 2006. https://www.emea.europa.eu/en/documents/mrl-report/lasalocid-sodium-extension-eggs-summary-report-committee-veterinary-medicinal-products_en.pdf

63. EMA (European Medicines Agency), 2012. Lasalocid (bovine species). European public MRL assessment report (EPMAR). EMEA/CVMP/504089/2010. https://www.ema.europa.eu/en/documents/mrl-report/lasalocid-bovine-species-european-public-mrl-assessment-report-epmar-cvmp_en.pdf

64. FAO (Food and Agriculture Organization of the United Nations), 2015. Online Edition: Residues of some veterinary drugs in foods and animals. Food safety and quality: Details (fao.org)

65. FAO/WHO (Food and Agriculture Organization/World Health Organization), 2015. Evaluation of certain veterinary drug residues in food. Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 997, Rome 17-25 November 2015. https://apps.who.int/iris/bitstream/handle/10665/204670/9789240695504_eng.pdf?sequence=1

66. FDA (Food and Drug Administration) CFR – Code of Federal Regulations Title 21 Volume 6 Part 556 Tolerances for Residues of New Animal Drugs in Food (21CFR556) CFR - Code of Federal Regulations Title 21 (fda.gov)

67. Government of Canada. List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods. August 26, 2022. List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods - Canada.ca

68. JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES Seventy-eighth meeting (Residues of veterinary drugs) Geneva, 5-14 November 2013 (JECFA/78/SC)

69. Kennedy, D.G., Blanchflower, W.J., O'Dornan, B.C. 1995. Development of an ELISA for lasalocid and depletion kinetics of lasalocid residues in poultry. Food Addit. Contam., 12:83-92.

70. Kennedy, D.G., Blanchflower, W.J., Hughes, P.J., McCaughey, W.J. 1996. The incidence and cause of lasalocid residues in eggs in Northern Ireland. Food Addit. Contam., 13(7):787-794.

71. Kennedy, D.G., Hughes, P.J., Blanchflower, W.J. 1998. Ionophore residues in eggs in Northern Ireland: incidence and cause. Food Additives and Contaminants, 15(5):535-541.
72. Martinez A., Jones C. K., Stark C. R., McKinney L. J., Behnke K. C., Paulk C. B. 2018. Evaluating flushing procedures to prevent nicarbazin carryover during medicated feed manufacturing, Animal Feed Science and Technology, 242:1-7, ISSN 0377-8401. https://doi.org/10.1016/j.anifeedsci.2018.04.017
73. Mortier, L., Huet, A.C., Charlier, C., Daeseleire, E., Delahaut, P, Van Peteghem, C. 2005. Incidence of residues of nine anticoccidials in eggs. Food Additives and Contaminants, 22(11):1120-1125.
74. NADA 096-298. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. 1995. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/342
75. NADA 107-996. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. 2002. Lasalocid (AVATEC®) plus Bacitracin methylene disalicylate (BMD®). https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/362
76. NADA 141-109. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. Combined use of AVATEC® and BACIFERM® in turkey feeds. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/638
77. NADA 141-488. New Animal Drug Application (NADA), FDA-HHS (Department of Health and Human Services, Food and Drug Administration), Freedom of Information Summary. Lasalocid type A medicated article and lincomycin type A medicated article. 2018 https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/3944
78. National Residue Survey (NRS), Department of Agriculture, Fisheries, and Forestry. Australian Government. eggs-residue-testing-annual-datasets-2021-22 (agriculture.gov.au)
79. Noser, J., Wenk, P., Sutter, A., Schneider, P. 2006. Kokzidiostatika in Eiern, Geflugel-fleisch under Futtermitteln: Eine Stichproben-Kontrolle uber den Nordwestschweizer Markt. Mitt Lebensm Hyg 97:107-120.
80. Olejnik, M., Szprengier-Juszkiewicz, T., Jedziniak, P. 2014. Distribution of semduramicin in hen eggs and tissues after administration of cross-contaminated feed. Food Additives and Contaminants: Part A, 31(8):1393-1398.
81. Roila, R., Branciari, R., Pecorelli, I., Cristofani, E., Carloni, C., Ranucci, D., Fioroni, L. 2019. Occurrence and residue concentration in feed and food of animal origin; human exposure assessment. Foods 8(10):477. Occurrence and Residue Concentration of Coccidiostats in Feed and Food of Animal Origin; Human Exposure Assessment (mdpi-res.com)
82. Rokka, M., Eerola, S., Perttila, U., Rossow, L., Venalainen, E., Valkonen, E., Valaja, J., Peltonen, K. 2005. The residue levels of narasin in eggs of laying hens fed with unmedicated and medicated feed. Molecular Nutrition and Food Research, 49(1):38-42.
83. Rokka, M., Jestoi, M., Peltonen, K. 2013. Trace level determination of polyether ionophores in feed. Biomed Research International, vol. 2013, Article ID 151363, 12 pages.
84. Strauch, W. 2002. Causes and control of carry-over and cross-contamination. Kraftfutter/Feed Magazine, 85(4):151-159 (Part 1). Kraftfutter/Feed Magazine, 85(6):239-249 (Part 2).
85. UK-VMD (United Kingdom-Veterinary Medicines Doctorate), 2022. Maximum residue limits in Great Britain. Maximum Residue Limits in Great Britain (publishing.service.gov.uk)
86. Vandenberge, V., Delezie, E., Huyghebaert, G., Delahaut, P., Pierret, G., De Backer, P., Croubels, S., Daeseleire, E. 2012. Transfer of the coccidiostats monensin and lasalocid from feed at cross-contamination levels to whole egg, egg white and egg yolk. Food Additives and Contaminants: Part A, 29(12):1881-1892.
87. Wong, R.Y., Roxburgh, J.W. 2010. Lasalocid awareness and sampling in Scotland. International Journal of Environmental Health Research, 20(3):159-169.
88. Young, R., Nunan, C. 2004. Too hard to crack? Eggs with drug residues. Report Four in The use and misuse of antibiotics in UK agriculture series. Soil association.

Attachment 1

Table: Compilation of residues data for nicarbazin measured in edible tissues from poultry fed nicarbazin medicated feed

Species	Feed level (mg/kg)	Dosing period (days)	LOD (mg/kg)	LOQ (mg/kg)	WHP (days)	Residue level (mg/kg)				Reference
						Liver	Kidney	Muscle	Skin/Fat	
Chicken	100 (WF or DL)	28	NS	0.001	9	0.2238±0.0742 (DL) 0.0237±0.0039 (WF)	-	0.0024±0.0003 (WF) 0.014±0.0084 (DL)	-	Cannavan and Kennedy, 2000
Chicken	13.5 (WF or DL)	32	NS	0.001	5	1.157 (DL) 0.992 (WF)	-	0.055 (DL) 0.028 (WF)	-	Cannavan and Kennedy, 2000
Chicken	125	28	NS	0.05 liver 0.1 kidney 0.025 muscle 0.025 skin/fat	1 (24 h)	9.249±1.804	3.007±1.095	2.110±0.506	2.327±0.372	EFSA 2010a
Chicken	125	42	0.03	0.1	1 (24 h)	14.4-21	2.8-5.4	1.4-2.2	1.6-3.0	Wood and Dowling, 1980; JECFA 1999
Chicken	125	49	0.02	0.1	1 (24 h)	2.69-9.12 6.62±1.08	-	0.85-1.23 0.98±0.088	0.66-0.99 0.88±0.042	Kramer, 1990; JECFA 1999; ANADA 200-027
Chicken	50 (+50 lasalocid)	42	NS	NS	1 (24 h)	8.57±1.432	3.51±1.12	1.64±0.294	1.95±0.257	EFSA 2021
*Chicken	50 (+50 monensin)	35	NS	0.1	0.25 (6 h)	8.331 (x+2SD)	1.514 (x+2SD)	1.182 (x+2SD)	1.723 (x+2SD)	EFSA 2017
*Chicken	55 (+55 monensin)	10	NS	NS	0.25 (6 h)	6.857±0.920	0.806±0.584	0.761±0.207	1.269±0.326	EFSA 2017; EFSA 2018b
*Chicken	45.4 (+45.4 narasin)	63	NS	2	0	7.6	-	<2	<2	NADA 138-952a

Species	Feed level (mg/kg)	Dosing period (days)	LOD (mg/kg)	LOQ (mg/kg)	WHP (days)	Residue level (mg/kg)				Reference
						Liver	Kidney	Muscle	Skin/Fat	
*Chicken	50 (+50 narasin)	35	NS	0.05 liver 0.1 kidney 0.025 muscle 0.025 skin/fat	0	9.19±0.956	4.29±1.034	1.61±0.149	2.04±0.479	NADA 138-952b; EFSA 2010b
*Chicken	70 (+70 narasin)	42	NS	0.02	0 (3h)	8.988±1.965	3.525±1.485	1.813±0.43	2.018±0.66	EFSA 2019
*Chicken	45 (+27 narasin + 4 lincomycin)	NS	NS	NS	0 (6h)	8.27±1.75	-	-	-	NADA 140-947
*Chicken	45 (+27 narasin + 50 bacitracin + 45.4 roxarsone)	21	NS	NS	0 (6 h)	10.4 (2.0-16.5)	-	-	-	NADA 141-112; NADA 141-113
*Chicken	50 (+50 narasin + 200 bacitracin	49	0.1	NS	0 (6h)	8.5±2.96	-	-	-	NADA 140-926; NADA 141-124; NADA 141-529
*Chicken	113 (+20 bambermycins, +50 roxarsone	48	NS	1	0	32.9±6.87	-	4.7±1.86	6.2±1.55	NADA 140-339
*Turkey	50 (+50 monensin) Turkeys	112 (16 wk)	0.01	0.1	0.25 (6 h)	0.276 (5 <LOQ, 1<LOD)	<LOQ	<LOD	<LOQ	EFSA 2017
*Turkey	109	112 (16 wk)	NS	1	0 (1 h)	1.22 (x+2SD)	<LOQ	<LOQ	<LOQ	EFSA 2018a
*Feeding studies with practical zero withdrawal times (less than 12 hours).										

Attachment 2

Table: Compilation of residue data for lasalocid measured in edible tissues from poultry fed lasalocid medicated feed.

Species	Feed level (mg/kg)	Dosing period (days)	Marker Residue	LOD (µg/kg)	LOQ (µg/kg)	WHP (days)	Residue level (µg/kg)				Reference
							Liver	Kidney	Muscle	Skin/Fat	
Chicken	125	7	Lasalocid A	20	NS	0 1 3 5 7	290 90 20 40 -	130 30 30 30 -	50 - - - -	340 70 40 40 -	EFSA, 2004
Chicken	90	14	Lasalocid	0.01 (Muscle) 0.09 (Liver)	0.02 (Muscle) 0.15 (Liver)	0 5 7	400 30 20	- - -	10 0.5 0.5	- - -	Kennedy et al., 1995
Chicken	125 then 132	34 then 21	Total Radioactivity	NS	NS	0 1 2 3 4 5	11930 2630 1720 1590 1370 1150	2480 360 230 < 200 < 200 < 200	610 60 30 < 200 < 200 < 200	1590, 860 220, 140 130, 60 < 200 < 200 < 200	EMA, 2004
Chicken	75 (+50 roxarsone)	56	lasalocid	NS	50	0 1 2 3	< LOQ < LOQ < LOQ < LOQ	< LOQ < LOQ < LOQ < LOQ	< LOQ < LOQ < LOQ < LOQ	370 ± 40 50 < LOQ < LOQ	NADA 141-488
Chicken	75 (+50 roxarsone + 2.0 lincomycin)	56	Lasalocid	NS	50	0 1 2 3	< LOQ < LOQ < LOQ < LOQ	< LOQ < LOQ < LOQ < LOQ	< LOQ < LOQ < LOQ < LOQ	360 ± 30 < LOQ < LOQ < LOQ	NADA 141-488

Species	Feed level (mg/kg)	Dosing period (days)	Marker Residue	LOD (µg/kg)	LOQ (µg/kg)	WHP (days)	Residue level (µg/kg)				Reference
							Liver	Kidney	Muscle	Skin/Fat	
Chicken	125 (+2.2 lincomycin + 50 roxarsone)	56	Lasalocid	NS	50	0	-	-	-	270 ± 110	NADA 141-488
						1	-	-	-	70 ± 20	
						2	-	-	-	< LOQ	
						3	-	-	-	< LOQ	
						4	-	-	-	< LOQ	
Chicken	125 (+55 bacitracin zinc)	42	Lasalocid	NS	15	0				428 ± 127	NADA 107-996
Turkey	125	14	Total Radioactivity	20	NS	0.33	3380 ± 570	430 ± 50	300 ± 10	300 ± 110	NADA 096-298
						1	1430	200	< LOD	160	
						2	1490	170	< LOD	110	
						3	1040	120	< LOD	100	
						4	1100	120	< LOD	140	
						5	870	80	< LOD	90	
Turkey	125 (+55 bacitracin zinc)	105	Lasalocid	NS	5	0.25	37.3 ± 30.4	-	-	-	NADA 141-109
Quail	90	27	Lasalocid A	NS	NS	0	-	-	-	298.3	EMA, 2004
						3	-	-	-	55	
						6	-	-	-	30.8	
						9	-	-	-	33.7	
Pheasant	132	7	Lasalocid A	NS	NS	0	28.5	-	-	30.7	EMA, 2004
NS – Not Specified.											

APPENDIX III
LIST OF PARTICIPANTS

Chair

Canada

Cole Enns

Health Canada

Vice-Chairs

Australia

James Deller

Department of Agriculture, Fisheries and Forestry

United States of America

Jonathan Greene

U.S. Food & Drug Administration

MEMBER COUNTRY/ORGANIZATION[1]

1. Argentina
2. Australia
3. Bahrain
4. Brazil
5. Canada
6. Chile
7. Cyprus
8. Denmark
9. Egypt
10. European Union
11. France
12. Germany
13. Guatemala
14. Honduras
15. India
16. Indonesia
17. Israel
18. Italy
19. Japan
20. Korea (Republic of)
21. Macedonia
22. Malaysia
23. Netherlands
24. New Zealand
25. Panama
26. Saudi Arabia
27. Senegal
28. Singapore
29. South Africa
30. Switzerland
31. Thailand
32. United Kingdom

OBSERVER[1]

1. IFIF
2. Health for Animals

[1] Please contact the focal point of the Member Country or Observer Organization for the details of the delegates. The list of Codex contact points for members is available from the Codex website at: http://www.fao.org/fao-who-codexalimentarius/about-codex/members/en/ http://www.fao.org/fao-who-codexalimentarius/about-codex/observers/observers/obs-list/en/