5.25 NATAMYCIN (300)

TOXICOLOGY

Natamycin (synonym pimaricin) is the WHO-approved nonproprietary name for (8E,14E,16E,18E,20E)-(1R,3S,5R,7R,12R,22R,24S,25R,26S)-22-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.05,7] octacosa-8,14,16,18,20-pentaene-25-carboxylic acid (IUPAC name), with the CAS number 7681-93-8. No ISO-approved name is available. Natamycin is a fungicide of the polyene macrolide class of antifungal antimicrobials used in the protection of foods and for treatment of human disease. In food production, natamycin can be used as surface treatment of cheeses and dried sausages. It is also used as for the topical treatment of fungal infections in humans and animals, such as mycotic keratitis. Natamycin was originally produced by *Streptomyces natalensis* in submerged aerobic batch culture fermentation but is also now known to be produced by several other *Streptomyces* species. Natamycin acts as fungicide by preventing the germination of fungal spores via binding to ergosterol located in fungal cellular membranes.

Natamycin has not been evaluated previously by the JMPR and was reviewed by the present Meeting at the request of the CCPR.

Natamycin was previously evaluated by twelfth, twentieth and fifty-seventh Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1968, 1976 and 2001 at the request of the Codex Committee on Food Additives and Contaminants (CCFA). The twentieth Meeting established an ADI of 0–0.3 mg/kg bw based on gastrointestinal effects in humans and application of a safety factor of 10. The 2001 Meeting reaffirmed its previous conclusion on the ADI.

This evaluation is based mainly on the study reports made available to the Meeting. A comprehensive literature search was also conducted. The articles relevant for the human health risk assessment were included in the evaluation and are described in the appropriate sections. Only a few submitted studies contained statements of compliance with GLP or were conducted according to internationally recognized guidelines (such as the USEPA/Federal Insecticide, Fungicide, and Rodenticide Act [FIFRA] or OECD). Most submitted studies pre-dated the GLP requirements or similar test guidelines. However, GLP status was not specifically checked. In general, studies were conducted with technical material; however, neither impurity profiles of the tested materials nor current specifications were provided. Hence, it could not be assessed whether the tested material was representative of the currently available commercial technical material.

In line with the JMPR mandate, only the impact of natamycin residues on human health was assessed in this evaluation. This included the assessment of the potential impact on the intestinal microbiota and possible induction of resistant microorganisms due to the presence of residues in the gastrointestinal tract. The possible impacts on workers involved in natamycin manufacture and operators using natamycin-containing products was not assessed.

Biochemical aspects

The absorption, excretion, tissue residues and metabolism of ^{14}C-labelled natamycin were investigated in rats and, to some extent, in dogs. The radioactivity in oral doses of 0.1 to 50 mg/kg bw administered to rats was readily excreted, with more than 95% of the dose eliminated in the faeces within 72 hours. Urinary elimination of administered radioactivity amounted to below 2%. Radioactivity eliminated as ^{14}C-labelled carbon dioxide was low. Sex differences were not investigated. Upon intraperitoneal administration, amounts excreted via faeces were 7-fold those excreted via urine. Based on comparisons of urinary amounts after oral and intraperitoneal administration, oral bioavailability was approximately 13% or less. In dogs, excretion via urine amounted to less than 4%, but based on the results of intravenous administration in dogs, amounts excreted in the bile may be assumed to be similar to that via urine. Total radioactivity in major organs and tissues were generally low.

Identities of natamycin-related metabolites were not investigated.

Livers of rats treated with oral doses of natamycin at 0.3, 1, 3 and 10 mg/kg bw per day for 6 days were isolated and the microsomes prepared and used to determine the activities of several cytochrome P450 enzymes (CYP). The lowest dose of natamycin (0.3 mg/kg bw per day for 6 days) had no significant effects on the activities of the hepatic cytochrome enzymes studied. For the 1, 3 and 10 mg/kg bw doses, significant dose-dependent decreases in the total hepatic cytochrome content and in the activities of aniline hydroxylase (associated with CYP2E1), aminopyrine *N*-demethylase, MROD (associated with CYP1A2), EROD (associated with CYP1A1) and PROD (associated with CYP2B1/2) enzymes was observed. Natamycin also produced a significant decrease in the 12- and 11-hydroxylation of lauric acid (associated with CYP4A subfamily).

Toxicological data

Acute toxicity of natamycin was determined after oral (rat LD_{50} > 2000 mg/kg bw), dermal (rat LD_{50} > 5050 mg/kg bw) and inhalation (rat LC_{50} > 2.39 mg/L) exposure. Natamycin induced slight and transient irritation in skin and eyes. In a mouse LLNA, no induction of skin sensitization was seen.

Short-term toxicity studies with oral administration were conducted in the rat and dog. Mainly body weights and feed intakes were affected in rats and dogs; diarrhoea was also observed in dogs.

In a 13-week dietary toxicity study, groups of male and female rats received diets containing natamycin at concentrations of 0, 125, 500 or 2000 ppm (equal to 0, 11, 42 and 204 mg/kg bw per day for males and 0, 12, 48 and 238 mg/kg bw per day for females, respectively; when considering feed scatter, the substance intakes were 10–30% lower). The NOAEL was 500 ppm (equal to 42 mg/kg bw per day) based on reduced body weights and clinical chemistry findings such as changes in alanine aminotransferase, urea, inorganic phosphorous, cholesterol and total protein at 2000 ppm (equal to 204 mg/kg bw per day).

In a 90-day dietary toxicity study, groups of two male and two female dogs received diets containing natamycin at concentrations of 0, 375 or 750 ppm (equivalent to 0, 12 and 25 mg/kg bw per day). The NOAEL was 375 ppm (equivalent to 12 mg/kg bw per day) based on lower body weight gains and increased number of days with diarrhoea at 12 mg/kg bw per day.

In a 2-year dietary toxicity study, groups of three male and three female dogs received diets containing natamycin at concentrations of 0, 125, 250 or 500 ppm (equivalent to 0, 3.1, 6.3 and 12.5 mg/kg bw per day). The NOAEL was 250 ppm (equivalent to 6.3 mg/kg bw per day) based on lower body weight gains in males at 12.5 mg/kg bw per day.

No reports on long-term toxicity or carcinogenicity studies in mice were available to the Meeting.

In a long-term toxicity and carcinogenicity study, groups of 35 male and 35 female rats received diets containing natamycin at concentrations of 0, 125, 250, 500 or 1000 ppm (equivalent to 0, 6, 12.5, 25 or 50 mg/kg bw per day) for up to 2 years. Body weights and feed consumptions were lower in the high-dose groups than in control groups. Due to the low number of animals per dose group and sex when compared with OECD guidelines 453 or 451, and the gross and microscopic examination of selected animals only, the results of this study do not contribute to the toxicological assessment of natamycin.

Considering the limitations in the available database on carcinogenicity, no conclusions can be drawn on the carcinogenic potential of natamycin.

Natamycin was tested for genotoxicity in a range of assays, both in vitro and in vivo. Natamycin tested negative in an Ames test and in an in vitro chromosomal aberration study, but increases in chromosomal aberrations and micronuclei were reported elsewhere. It is noted that no report on in vitro mammalian cell gene mutation assay was available. A summary report on the assessment of the veterinary drug natamycin listed GLP-compliant studies on mutagenicity that included Ames test, mouse LLNA and chromosomal aberration assay in Chinese hamster ovary cells, which gave negative results; however, the Meeting had no access to these studies. Follow-up in vivo

studies on clastogenicity/aneugenicity, which gave both positive and negative results, had serious limitations.

The Meeting concluded that the available database on the in vivo and in vitro genotoxicity of natamycin was inadequate to draw a clear conclusion on genotoxicity.

In view of the limitations in the available database on carcinogenicity and genotoxicity, the Meeting determined that no conclusions can be drawn on the carcinogenic risk to humans from the diet.

Groups of male and female rats were fed diets containing natamycin at concentrations adjusted to maintain target dose levels of 0, 5, 15, 50 and 100 mg/kg bw per day (achieved dose levels were not reported) for three generations. The NOAEL for parental toxicity was 15 mg/kg bw per day based on lower body weights at 50 mg/kg bw per day. The NOAEL for offspring toxicity was 5 mg/kg bw per day based on lower pup weights at 15 mg/kg bw per day. The NOAEL for reproductive toxicity was 50 mg/kg bw per day based on low pre- and postnatal survival at 100 mg/kg bw per day.

Groups of female rats from the second litters of the F_1 generation of a three-generation study of reproductive toxicity were reared to maturity on control diets and mated with untreated males. The F_{1b} females were given the same dose of natamycin as their parents (0, 5, 15, 50 or 100 mg/kg bw per day) by gastric intubation during gestation days 6–15; they were killed and examined on gestation day 20. The NOAELs for maternal toxicity and embryo/fetal toxicity were 100 mg/kg bw per day based on the absence of adverse effects up to the highest dose level tested.

In a developmental toxicity study, groups of female rabbits were treated with aqueous suspensions of natamycin at doses of 0, 5, 15 or 50 mg/kg bw per day via gavage during gestation days 6–18; they were killed and examined on day 29. The NOAEL for maternal toxicity was 50 mg/kg bw per day based on the absence of adverse effects up to the highest dose level tested. The NOAEL for embryo/fetal toxicity was 5 mg/kg bw per day based on the increased incidence of extra sternebrae and decreases in pup body weight of equivocal toxicological relevance at 15 mg/kg bw per day.

The Meeting concluded that the available studies with natamycin did not indicate teratogenic potential.

No reports from specific neurotoxicity studies were available to the Meeting. The submitted 90-day rat study did not indicate neurotoxic potential based on the absence of clinical signs.

The Meeting concluded that the limited available studies with natamycin did not show evidence of neurotoxicity.

No reports from specific immunotoxicity studies were available to the Meeting.

Microbial aspects

Data from in vitro minimum inhibitory concentrations (MIC), susceptibility and selectivity of natamycin resistance were available.

The Meeting concluded that the amounts of natamycin, as residues, were unlikely to induce resistance in gastrointestinal microbiota or colonization barrier disruption.

The Meeting considered that the limited available database, which did not raise specific health concerns, needs to be balanced against the long history of use and the data necessary for a thorough assessment of pesticide residues. The Meeting considered the available data insufficiently robust for the purpose of establishing health-based guidance values because of the limitations of the present database on natamycin (design of the animal studies, limited number of animals, unclear genotoxicity results, lack of adequate carcinogenicity studies) and because of the inadequate descriptions in some of the studies. The Meeting was aware that further toxicological data on natamycin were available to other agencies.

The Meeting concluded that the available database on natamycin was inadequate to characterize the potential hazards to the general population, including fetuses, infants and children, from natamycin residues from its use as a pesticide.

Human data

No effects were reported on the health of workers involved in the manufacture or use of natamycin. No information on accidental or intentional poisoning in humans were available.

No effects were reported in persons who received dermal or ocular natamycin treatment. Nausea, vomiting and diarrhoea were described in patients treated with oral natamycin.

Toxicological evaluation

The Meeting did not establish an ADI or an ARfD due to the inadequate database available to the Meeting.

A toxicological monograph was prepared.

Information that would be useful for the continued evaluation of the compound

Information on the genotoxic potential, information on the carcinogenic potential, results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to natamycin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Indications of low exposure
Dermal absorption	No data
Distribution	No conclusive data
Potential for accumulation	No conclusive data
Rate and extent of excretion	No conclusive data, excretion mainly via faeces and in lower amounts via urine
Metabolism in animals	No data
Toxicologically significant compounds in animals and plants	No data
Acute toxicity	
Rat, LD_{50}, oral	>2 000 mg/kg bw
Rat, LD_{50}, dermal	>5 050 mg/kg bw
Rat, LC_{50}, inhalation	>2.39 mg/L (4 h exposure, nose-only)
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Slightly irritating
Mouse, dermal sensitization	Non-sensitizing (LLNA)
Short-term studies of toxicity	
Target/critical effect	Low body weight gain, diarrhoea
Lowest relevant oral NOAEL	6.3 mg/kw bw per day (dog)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No data

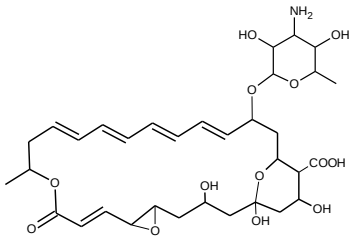
Lowest relevant NOAEL	No data
Carcinogenicity	No data to conclude on the carcinogenic potential
Genotoxicity	
	Contradictory results in vitro and in vivo (micronucleus/chromosomal aberration assay); no sufficient database to conclude on the genotoxic potential
Reproductive toxicity	
Target/critical effect	Low body weight gain in parental animals, low pre- and postnatal survival, low pup weight
Lowest relevant parental NOAEL	15 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	5 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	50 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Low pup weight, increased incidences of extra sternebrae
Lowest relevant maternal NOAEL	50 mg/kg bw per day[a] (rabbit)
Lowest relevant embryo/fetal NOAEL	5 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data; no indication of neurotoxic effects in 90-d rat study
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No specific in vivo data
Human data	
	No adverse health effects reported in manufacturing plant personnel or persons with dermal or ocular natamycin-treatment
	Nausea, vomiting and diarrhoea in patients with oral natamycin-treatment
Microbial aspects	
	Amounts of natamycin as residues were unlikely to induce resistance in gastrointestinal microbiota and colonization barrier disruption

[a] Highest dose tested.

RESIDUE AND ANALYTICAL ASPECTS

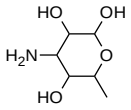
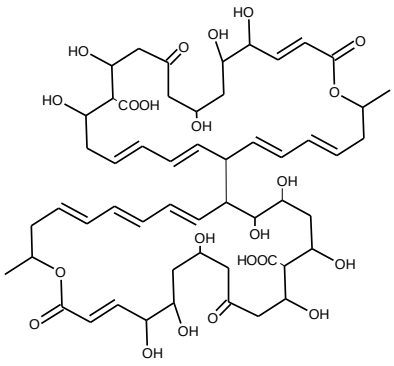
Residue and analytical aspects of natamycin were considered for the first time by the present Meeting. The residue evaluation was scheduled for the 2017 JMPR by the 48th Session of the CCPR. The Meeting noted that natamycin is used as a preservative in sausages and cheese and other dairy products and that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a maximum ADI for natamycin of 0.3 mg/kg bw in 1976 and confirmed that ADI in 2006. In addition, natamycin is used to treat fungal keratitis and is on the WHO's List of Essential Medicines.

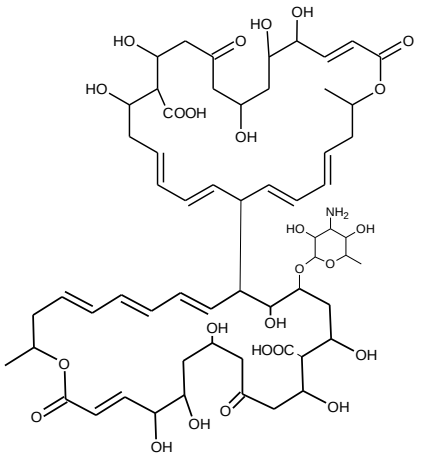
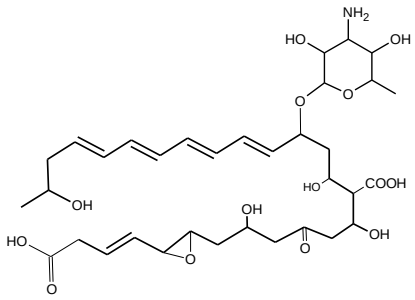
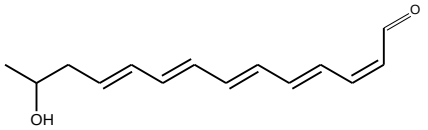
Natamycin is a contact fungistat belonging to the polyene macrolide class of compounds. The pesticidal mode of action is binding to ergosterol in the cell membrane resulting in prevention of fungal spore germination. The uses under consideration by the Meeting are application to mushrooms and post-harvest application to fruits. The Meeting received information on rat metabolism of natamycin, as well as acid and alkaline hydrolysis and UV photolysis. Data depicting the metabolism of natamycin in plants, fungi, livestock, and soils were not provided. Limited residue trial data, analytical methods data, storage stability data, and processing data were provided. Livestock feeding studies were not available.



Natamycin (8*E*,14*E*,16*E*,18*E*,20*E*)-(1*R*,3*S*,5*R*,7*R*,12*R*,22*R*,24*S*,25*R*,26*S*)-22-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

In this appraisal, the following abbreviated names were used for metabolites.

Identifier	Chemical Structure
Micosamine 4-amino-6-methyltetrahydro-2*H*-pyran-2,3,5-triol	
Natamycinolidediol	

Identifier	Chemical Structure
Aponatamycin	
Natamycoic acid	
13-Hydroxytetradeca-2,4,6,8,10-pentaenal	
Acetone	
Acetaldehyde	
Ammonia	NH_3

Plant and animal metabolism

The Meeting did not receive metabolism studies for natamycin in laboratory animals, plants (primary or rotational), fungi, or livestock.

Environmental fate in soil and water

The Meeting received information on acid and alkaline hydrolysis, and aqueous photolysis. The information that was provided is from a published book chapter and not from studies conducted according to guidelines. Nevertheless, the information is useful for characterizing the expected behaviour of natamycin under hydrolytic and photolytic conditions.

Under acid conditions, natamycin forms an unstable aglycone molecule which can dimerize with another natamycin aglycone to form natamycinolidediol or combine with an intact natamycin molecule to form aponatamycin. Under alkaline conditions, natamycin undergoes saponification to form natamycoic acid, which may undergo additional breakdown to form aldehydes, ketones, and ammonia.

Ultraviolet irradiation causes natamycin to become inactive as a fungistat. The process is believed to be via oxidation of the tetraene structure leading to formation of polymers similar to those observed during acid hydrolysis.

Methods of analysis

The Meeting received description and validation data for analytical methods for residues of natamycin in tropical fruits (citrus and pineapple) and mushrooms.

For all of the submitted methods, residues of natamycin are extracted with methanol and analysis for residues is by LC-MS/MS. For citrus flesh only, the methanol extracts undergo clean-up by solid-phase extraction prior to analysis. The methods were validated to an LOQ of 0.01 mg/kg, as a lower limit of method validation, in mushroom, pineapple, and citrus flesh, and to 0.1 mg/kg in mushroom casing and compost and citrus whole fruit and peel. Suitable ion transitions are available for residue quantification and confirmation. Mean recoveries from edible commodities (mushrooms and fruits) ranged from 70% to 108% with a maximum relative standard deviation of 15%. The methods have been shown to be suitable for analysis of natamycin in high-acid and high-water commodities.

Stability of residues in stored analytical samples

The Meeting received data on the stability of residues of natamycin in mushroom, pineapple, and citrus matrices. Homogenized control samples of each matrix were fortified with natamycin and placed into frozen storage (≤ -10 °C) concurrently with residue trial samples.

Residues of natamycin were stable in mushrooms (frozen, temperature not specified) for at least 93 days, in pineapple commodities for at least 48 days, and in citrus commodities for at least 78 days. The Meeting noted that the mushroom analysis did not include 0-day samples to confirm the fortification level.

Definition of the residue

Plants/fungi

Except for the use on mushrooms, the uses under consideration by the Meeting are post-harvest uses. As such metabolism under typical agricultural field conditions is not particularly germane to determining residue definitions.

Natamycin undergoes breakdown under hydrolytic and photolytic conditions but is considered to be stable under dry, dark conditions. For the post-harvest uses being considered by the Meeting, natamycin is not expected to undergo significant degradation on the surface of the fruits. Residue decline data for citrus support this contention; however, residue decline data from pineapple show an approximately 2-fold reduction in residues between Day 0 and Day 7 after treatment, followed by a residue plateau from post-treatment days 7–21 (study duration 0–21 days). As for mushrooms, mushroom compost and casing soil are typically maintained at near-neutral pH levels[1] and cultivation is generally under low-light conditions. Given the information on hydrolysis and photolysis, these conditions are likely to minimize breakdown of natamycin.

Analytical methods are available that are suitable for analysis of natamycin.

The Meeting agreed that natamycin is a suitable marker for compliance with MRLs in citrus fruits and mushrooms. Furthermore, the Meeting agreed that the residue definition for assessing dietary risk from these commodities is natamycin.

Definition of the residue for plant commodities and fungi (for compliance with the MRL and for dietary risk assessment): *Natamycin*

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[1] Allison, W. H. and Kneebone, L. R., 1963, *Influence of Compost pH and Casing Soil pH on Mushroom Production*, International Society for Mushroom Science, Volume 5, Part 1.

Results of supervised residue trials on crops

The Meeting received supervised trial data reflecting application of natamycin to growing mushrooms and post-harvest application to citrus fruits and pineapple.

Labels for end-use products containing natamycin were available from Canada describing the registered use on mushrooms and from the United States of America describing the registered uses on citrus fruits, pineapple, and mushrooms.

For all trials, residues were determined by the methods referenced above (methanol extraction, LC-MS/MS analysis). Analyses were completed within ca. one month for pineapple and mushrooms and within ca. 3 months for citrus. The available storage stability data support the storage durations and conditions from the residue studies.

Citrus fruits

Natamycin is registered in the US for post-harvest use on citrus (including calamondin, citrus citron, grapefruit, kumquat, lemon, lime, mandarin, oranges, and pummelo). The USA GAP is for a single application via in-line dip, drench, or aqueous or fruit-coating spray at a concentration of 0.99 g ai/L. The label does not specify a holding period following treatment; therefore, the Meeting assumed that treated fruits could enter commerce on the day of treatment.

Three supervised trials were conducted according to the USA GAP on each of grapefruit, lemon, and orange, with application made by dipping into an aqueous treatment solution. Residues zero days after application were:

Grapefruit (n=3): 0.81, 1.0 (2) mg/kg,

Lemon (n=3): 1.5, 1.7, 1.8 mg/kg, and

Orange (including mandarin; n=3): 1.3, 1.8, and 2.3 mg/kg.

Based on the similarity of the residue levels, the Meeting decided to combine the data for making residue estimates (n=9):0.81, 1.0 (2), 1.3, 1.5, 1.7, 1.8, 1.9, 2.3 mg/kg.

The Meeting estimated a maximum residue level for residues of natamycin in citrus fruits of 5 (Po) mg/kg

Residues of natamycin in citrus flesh from that same study were (n=9): 0.019, 0.031, 0.058, 0.060, 0.064, 0.076, 0.0800, 0.096, and 0.11 mg/kg.

The Meeting estimated an STMR of 0.064 mg/kg and an HR of 0.11 mg/kg.

Assorted tropical and sub-tropical fruit – inedible peel

Pineapple

Natamycin is registered in the USA for post-harvest use on pineapple. The US GAP is for a single application via dip, pour, or cascade in wax at a concentration of 11 g ai/L. The label specifies applying natamycin to the peduncle after the initial application has dried.

The label does not specify a holding period following treatment; therefore, the Meeting assumed that treated pineapples could enter commerce on the day of treatment. Only one independent trial reflected GAP and reporting residues on the day of treatment was provided. The residue was:

Pineapple (n=1): 0.86 mg/kg.

The Meeting determined that there are insufficient data to estimate a maximum residue level for pineapple.