

# **NITROFURANS IN PRAWNS**

## **A Toxicological Review and Risk Assessment**

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## SUMMARY

### Introduction

Nitrofurans are synthetic broad-spectrum antimicrobial agents used in some countries in human and veterinary medicine. There are 4 main nitrofurans referred to in the scientific literature, namely, furazolidone, furaltadone, nitrofurantoin and nitrofurazone with all four nitrofurans having marker metabolites of 3-amino-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-1,3-oxazolidinone (AMOZ), 1-aminohydantoin (AHD) and semicarbazide (SEM), respectively. Nitrofurans have been prohibited from use in food-producing animals in most countries due to public health and safety concerns, particularly in relation to the carcinogenic potential of either the parent compounds or their metabolites.

In October 2003, the Australian Prawn Farmers Association (APFA) provided laboratory results to FSANZ indicating the presence of nitrofurans in certain prawns imported into Australia. The prawn samples were analysed for all four nitrofurans. AOZ was detected in a number of prawn samples - no other nitrofurans were detected. The Australian Quarantine and Inspection Service (AQIS) and Queensland Health Department reported additional data on nitrofurans in prawns for the period December 2003 to April 2004. This data showed levels of AOZ residues in the range 1.1-40 µg/kg. Although the majority of nitrofurans detected were AOZ, residues of AMOZ (one detection at 2.2 µg/kg) and SEM (one detection at 8.9 µg/kg in dried prawns only) were also detected. No residues of AHD were found in any of the samples.

FSANZ's has undertaken a risk assessment to determine whether there were any public health and safety concerns from residues of nitrofurans in prawns with a particular focus on furazolidone and its metabolite AOZ, as this was the metabolite that was most frequently found in prawn samples.

### Hazard assessment

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated furazolidone and nitrofurazone in 1993. JECFA's concluded that on the basis of the positive effects of furazolidone in genotoxicity tests *in vitro* and the increased incidence of malignant tumours in mice and rats, that furazolidone was a genotoxic carcinogen.

JECFA also concluded that nitrofurazone produced tumours in rats and mice but that these were benign and restricted to endocrine organs and the mammary gland, although nitrofurazone was genotoxic *in vitro*. JECFA did not establish an acceptable daily intake (ADI) for furazolidone or nitrofurazone.

## Dietary Exposure Assessment

A dietary exposure assessment for nitrofurans in prawns was undertaken based upon the dietary survey data for Australia from the 1995 National Nutrition Survey (NNS).

Using the lower and upper bound<sup>1</sup> mean concentration levels for AOZ residues from the Queensland Health and AQIS data and consumption figures for mean and high consumers of prawns, the dietary exposure to AOZ was determined as follows:

*Estimated dietary exposures to AOZ metabolites*

Metabolite	Estimated consumer mean dietary exposure		Estimated consumer 95 <sup>th</sup> percentile dietary exposure	
	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)
AOZ	0.060 (0.0009)	0.129 (0.0019)	0.200 (0.003)	0.430 (0.0064)

\* Mean body weight for Australians from the 1995 NNS for respondents aged 2 years and above = 67kg

## Risk Characterisation

The JECFA review of the toxicity data found that furazolidone induced a variety of tumours in rats and was positive in *in vitro* genotoxicity tests. No conclusion could be made regarding *in vivo* genotoxicity - one *in vivo* mouse micronucleus test was negative while the other was equivocal. The available data indicated that furazolidone induces malignant tumours (mammary adenocarcinomas, basal cell carcinomas and neural astrocytomas) in rats at doses of 25 mg/kg bw/day and above. A range of benign tumours was also observed. On the basis of this data, furazolidone should be regarded as a potential carcinogen in humans, although there is insufficient data to conclude that the tumour formation is initiated through a genotoxic mechanism. Whether there is a threshold for the observed tumour formation therefore remains unclear.

There are no long-term dietary studies on AOZ that would enable a direct comparison between the dose at which AOZ itself might produce tumours in animals and the level of human dietary exposure of AOZ. However, the risk associated with exposure to AOZ was characterised by determining the margin of exposure between the known levels of AOZ residues in prawns for mean and high consumers of prawns and the level of the parent compound furazolidone shown to cause tumours in animal studies. In addition, a comparison has been made between the levels of dietary exposure to AOZ and the acceptable daily intake (ADI) previously established in Australia.

When the dietary exposure for high consumers of prawns (upper bound) was compared to the dose shown to cause tumours in animal studies, there was an approximate 4 million-fold difference. At this level of dietary exposure, the risk of tumour formation from exposure to AOZ is likely to be extremely small, even in the absence of a threshold for tumour formation. However, the mean exposure level is a more realistic estimate of long-term exposure and if

<sup>1</sup> Refer to Attachment 1 for further details on lower and upper bound mean concentration levels and how they were calculated.

this figure (upper bound) is used in the above comparison, then the margin between dietary exposure and the dose causing tumours in animals increases to 12 million. When a comparison of the estimated exposure to AOZ residues for mean and high consumers of prawns to the ADI previously established in Australia was undertaken (using a worst-case exposure estimate for high consumers) the exposure is 1.5% of the ADI, again indicating a very low level of risk.

It is concluded that on the basis of information available to FSANZ, even with a worst-case scenario, the public health and safety risk from nitrofurans residues in prawns is considered very low.

## INTRODUCTION

Nitrofurans are synthetic broad-spectrum antimicrobial agents used in some countries in human and veterinary medicine. There are 4 main nitrofurans referred to in the scientific literature, namely, furazolidone, furaltadone, nitrofurantoin<sup>2</sup> and nitrofurazone.

The use of these four nitrofurans in food producing animals can be detected by analysing for their metabolites as residues in food.

The respective residues are as follows:

Parent compound	Metabolite (residue)
Furazolidone	3-amino-oxazolidinone (AOZ)
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ)
Nitrofurantoin	1-aminohydantoin (AHD)
Nitrofurazone	Semicarbazide (SEM).

Nitrofurans have been prohibited from use in food-producing animals in most countries due to public health and safety concerns, particularly in relation to the carcinogenic potential of either the parent compounds or their metabolites. Australia prohibited the use of nitrofurans in late 1992 and the EU prohibited the use of nitrofurans in food-producing animals in 1995.

### Chemical Properties

The basic chemical structures of the individual nitrofurans and their marker metabolites are detailed in Appendix 1 of **Attachment 2**.

### Previous Acceptable Daily Intake (ADI) established by Australia

Prior to 1992, furazolidone was registered for use in Australia. An ADI of 0.0004 mg/kg bw was established based on a no-observed-effect level (NOEL) of 0.75 mg/kg bw/day in a long-term study in Sprague-Dawley rats<sup>3</sup>, using a 2000-fold safety factor.

The Office of Chemical Safety within the Australian Department of Health and Aging establishes and reviews the ADIs for agricultural and veterinary chemicals in Australia. The ADI for furazolidone was withdrawn in December 2003 in line with the revised policy to remove from the official ADI list chemicals no longer in use in Australia.

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<sup>2</sup> Nitrofurantoin can be used to treat urinary cystitis in adults at doses not exceeding 400 mg/day (MIMS Annual 1993).

<sup>3</sup> This study is referenced in the WHO (1993) evaluation of furazolidone.

## DETECTION OF NITROFURAN RESIDUES IN PRAWNS

In October 2003, the Australian Prawn Farmers Association (APFA) provided laboratory results to FSANZ indicating the presence of nitrofurans in certain prawns imported into Australia.

The prawn samples were analysed for the following nitrofurans:

- Semicarbazide (SEM);
- 3-amino-oxazolidinone (AOZ);
- 1-aminohydantoin (AHD); and
- 3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ).

AOZ was detected in a number of prawn samples - no other nitrofurans were detected. These results suggested that the only nitrofurans used in the production of these prawns was furazolidone.

Additional data for the period December 2003 to April 2004 have been provided by the Australian Quarantine and Inspection Service (AQIS) and Queensland Health. This data showed levels of AOZ residues in the range 1.1-40 µg/kg (see **Table 1, Attachment 1**).

In this data set, it was noted that although the majority of nitrofurans detected were AOZ, residues of AMOZ<sup>4</sup> (one detection at 2.2 µg/kg) and SEM<sup>5</sup> (one detection at 8.9 µg/kg in dried prawns only) were also detected. No residues of AHD were found in any of the samples.

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<sup>4</sup> Queensland Health results

<sup>5</sup> AQIS results

## **TOXICOLOGICAL REVIEWS ON FURAZOLIDONE AND NITROFURAZONE**

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated furazolidone and nitrofurazone in 1993 (WHO, 1993).

### **Furazolidone**

JECFA concluded the following:

*On the basis of the positive effects of furazolidone in genotoxicity tests in vitro and the increased incidence of malignant tumours in mice and rats, the Committee concluded that furazolidone was a genotoxic carcinogen. Since the drug is rapidly and extensively metabolised, the Committee also considered information on metabolites of furazolidone. Although a large number of postulated metabolites produced negative results in genotoxicity tests, it was noted that only a few of these had been either identified or quantified in rats and pigs. Furthermore, the Committee concluded that insufficient data were available on the nature and toxic potential of compounds released from the bound residues. Because of the genotoxic and carcinogenic nature of furazolidone and the above-mentioned deficiencies with respect to the data on the metabolites, the Committee was unable to establish an ADI.*

JECFA's conclusion that furazolidone was a genotoxic carcinogen is based on limited genotoxicity data (only *in vitro* data) and FSANZ would not necessarily agree with this conclusion in the absence of positive *in vivo* genotoxicity studies with furazolidone.

### **Nitrofurazone**

JECFA concluded that nitrofurazone produced tumours in rats and mice but that these were benign and restricted to endocrine organs and the mammary gland. JECFA also concluded that nitrofurazone was genotoxic *in vitro*. It was noted that the International Agency for Research on Cancer (IARC) had concluded that, in relation to its potential carcinogenicity, there was limited evidence in animals and that there was inadequate evidence in humans (IARC, 1990). JECFA did not establish an acceptable daily intake (ADI) or maximum residue limit (MRL) for nitrofurazone.

## **FSANZ'S APPROACH TO THE RISK ASSESSMENT OF NITROFURAN RESIDUES IN PRAWNS**

FSANZ's risk assessment has focused on furazolidone and its metabolite AOZ rather than nitrofurazone or the other parent compounds (furaltadone and nitrofurantoin) for the following reasons:

- The majority of residues detected in samples of prawns were AOZ (**Attachment 1**) with only one detection each of AMOZ and SEM in the prawns sampled;
- AOZ is a metabolite produced from the use of furazolidone in animals;

- The toxicological data available has been conducted largely on furazolidone rather than its metabolite, AOZ;
- Furazolidone administration via the oral route produced malignant tumours in animals whereas nitofurazone produced benign tumours following oral administration. Therefore, the risk associated with furazolidone metabolites is likely to be greater;
- There is little toxicological data available on furaltadone which could be used to determine the risk associated with AMOZ residues (the metabolite of furaltadone); and
- Nitrofurantoin metabolites (AHD) have not been found in any of the prawn samples.

No original toxicological data on furazolidone were reviewed during this assessment, as a comprehensive toxicological report from the Joint Expert Committee on Food Additives (JECFA), an internationally recognised Committee, was available (**refer to Attachment 2**).

## DIETARY EXPOSURE ASSESSMENT

Dietary exposure assessment determines the amount of a chemical to which a population is exposed through the consumption of food and beverages. Dietary exposure assessment is conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data.

The dietary exposure assessment for nitrofurans was based upon the dietary survey data for Australia from the 1995 National Nutrition Survey (NNS). This survey has results for 13, 858 people aged 2 years and above, using a 24-hour food recall method.

There were 384 consumers of prawns on the day of the National Nutrition Survey (3% of the total number of respondents 13, 858). These consumption figures include where prawns were eaten as prawns, and/or where prawns were consumed as an ingredient in a mixed food (eg. prawn cocktail, seafood soup etc.).

Consumption of prawns for mean and high level consumers based on the above survey is as follows:

Mean consumption (2 years and above)	95 <sup>th</sup> percentile consumption (2 years and above)
75g/day	250g/day

Using the lower and upper bound<sup>6</sup> mean concentration levels for AOZ residues from the Queensland Health and AQIS data and the above consumption figures for prawns, the dietary exposure to AOZ was determined as follows:

<sup>6</sup> Refer to Attachment 1 for further details on lower and upper bound mean concentration levels and how they were calculated.

## Estimated dietary exposures to AOZ metabolites

Metabolite	Estimated consumer mean dietary exposure		Estimated consumer 95 <sup>th</sup> percentile dietary exposure	
	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)
AOZ	0.060 (0.0009)	0.129 (0.0019)	0.200 (0.003)	0.430 (0.0064)

\* Mean body weight for Australians from the 1995 NNS for respondents aged 2 years and above = 67kg

A full report on the dietary exposure assessment for AOZ and the other residues is at **Attachment 1**.

## RISK CHARACTERISATION OF NITROFURAN RESIDUES IN PRAWNS

The JECFA review of the toxicity data found that furazolidone induced a variety of tumours in rats and was positive in *in vitro* genotoxicity tests. No conclusion could be made regarding *in vivo* genotoxicity - one *in vivo* mouse micronucleus test was negative while the other was equivocal. The available data indicated that furazolidone induces malignant tumours (mammary adenocarcinomas, basal cell carcinomas and neural astrocytomas) in rats at doses of 25 mg/kg bw/day and above. A range of benign tumours was also observed.

On the basis of this data, furazolidone should be regarded as a potential carcinogen in humans, although there is insufficient data to conclude that the tumour formation is initiated through a genotoxic mechanism. Whether there is a threshold for the observed tumour formation therefore remains unclear.

Due to the lack of available data on the toxicity of the metabolites, FSANZ has adopted a cautious approach and assumed that the toxicity of the metabolite AOZ detected in prawns is the same as the toxicity of furazolidone. The risk associated with exposure to AOZ has been characterised by determining the margin of exposure between the known levels of AOZ residues in prawns for mean and high consumers of prawns and the level of the parent compound furazolidone shown to cause tumours in animal studies. In addition, a comparison has been made between the levels of dietary exposure to AOZ and the ADI previously established in Australia.

The margin of exposure and a comparison to the previous ADI for both mean and high consumers is as follows:

### Mean Consumers

Exposure to	Dose inducing	Margin of	% of ADI <sup>7</sup>
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<sup>7</sup> Previously established by Australia

<b>AOZ residues (µg/kg bw/day)</b>	<b>tumours in rats (mg/kg bw/day)</b>	<b>exposure</b>	<b>(0.0004 mg/kg bw)</b>
0.0019 (Upper bound)	25	12,500,000	0.5%
0.0009 (Lower bound)	25	25,000,000	0.25%

### High Consumers

<b>Exposure to AOZ residues (µg/kg bw/day)</b>	<b>Dose inducing tumours in rats (mg/kg bw/day)</b>	<b>Margin of exposure</b>	<b>% of ADI (0.0004 mg/kg bw)</b>
0.0064 (Upper bound)	25	4, 200,000	1.5%
0.0030 (Lower bound)	25	8, 300,000	0.75%

When the dietary exposure for high consumers of prawns (upper bound) was compared to the dose shown to cause tumours in animal studies, there was an approximate 4 million-fold difference. At this level of dietary exposure, the risk of tumour formation from exposure to AOZ is likely to be extremely small, even in the absence of a threshold for tumour formation.

The estimated dietary exposure for high level consumers is very conservative since consumers are highly unlikely to consume prawns every day at a high level. The 95<sup>th</sup> percentile consumption figure is therefore a highly conservative estimate of exposure. The mean exposure level is a more realistic estimate of long-term exposure and if this figure (upper bound) is used in the above comparison, then the margin between dietary exposure and the dose causing tumours in animals increases to 12 million.

The risk has also been characterised by comparing the estimated exposure to AOZ residues for mean and high consumers of prawns to the ADI previously established in Australia. Using a worst-case exposure estimate for high consumers, the exposure is 1.5% of the ADI, again indicating a very low level of risk.

FSANZ did not consider it necessary to characterise the risk associated with residues of AMOZ or SEM for the following reasons:

- There were only a single detection of each of these residues found in prawns (1/85 for SEM and 1/50 for AMOZ);
- Nitrofurazone (subsequent metabolite SEM) has a lower carcinogenic potential than furazolidone; and
- Limited toxicological data is available on furaltadone or its metabolite, AMOZ.

## **CONCLUSION**

The residues for AOZ found in prawns are considered to arise from the use of furazolidone in prawn production. There is a wide margin of exposure between the dietary exposure of AOZ residues from prawns for the highest consumer (95<sup>th</sup> percentile) and the dose at which the parent compound furazolidone caused cancer in animals. In addition, the level of exposure to furazolidone residues for high consumers was 1.5% of the ADI previously established in Australia.

There are no long-term dietary studies on AOZ that would enable a direct comparison between the dose at which AOZ itself might produce tumours in animals and the level of human dietary exposure of AOZ. A cautious approach was therefore used in assuming that the toxicity of the metabolite detected in prawns is the same as the toxicity of furazolidone. Even with this conservative assumption, the margin of exposure is very high.

On the basis of information available to FSANZ, even with a worst-case scenario, the public health and safety risk from nitrofurans residues in prawns is considered very low.

## **REFERENCES**

WHO (1993) Evaluation of certain veterinary drug residues in food. Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 832, p32 to 40.

International Agency for Research on Cancer (1990) IARC Monographs on the evaluation of carcinogenic risks to humans, 50, 195-209.

### **Dietary exposure assessment report**

A dietary exposure assessment was conducted to determine potential exposure of the Australian population to nitrofurans in prawns based on analytical data available to FSANZ.

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data. The assessment was conducted using prawn consumption data based on the 1995 Australian National Nutrition Survey (NNS), derived using FSANZ's dietary modelling computer program, DIAMOND. The 1995 NNS surveyed 13 858 people aged 2 years and above. The NNS used a 24-hour food recall methodology.

#### **Prawn consumption data**

There were 384 consumers of prawns on the day of the NNS. This includes where prawns were eaten on their own or as an ingredient in a mixed food, such as a seafood soup.

The mean consumption for consumers of prawns was 75 grams per day, and the 95<sup>th</sup> percentile consumption for consumers was 250 grams per day.

A Food Frequency Questionnaire (FFQ) was also conducted as a part of the 1995 NNS on respondents aged 12 years and above. In the FFQ, prawns were considered under 'other seafood'. The FFQ data shows that 'other seafood' is only eaten on a daily basis by 0.2% of the population. 29% of the population consume 'other seafood' once per month and the majority of the population (66%) consume 'other seafood' less than once per month or less. This data indicates that prawns are not a commonly consumed food, and the consumption figures outlined above would not be consumed daily by the majority of the population. Resulting estimated daily dietary exposures to nitrofurans in prawns are therefore only representative of exposures on days consumers eat prawns.

#### **Nitrofurans concentration levels in prawns**

The levels of nitrofurans in prawns used for the dietary exposure assessment were from two sources; the Australian Quarantine and Inspection Service (AQIS) and Queensland Health. A summary of the data, the detections found from each source can be found in Table 1.

The first set of data, collected by AQIS from 8 December 2003 to 16 April 2004, was for samples of imported prawns. AQIS disallows shipments of prawns to enter Australia if nitrofurans are detected, however, only 25% of cooked prawns and 5% of other prawns coming into the country are tested at the present time. This means that there may be some prawns, with residues levels similar to those detected by AQIS during their testing, entering the country and the food supply simply because they are not selected for testing. Eighty-six samples of prawns were analysed by AQIS including cooked prawns, uncooked prawns and dried prawns.

The second set of analytical data available to FSANZ was from a survey conducted by Queensland Health. Fifty-two individual retail samples were randomly selected and analysed.

The Queensland survey is representative of what prawns are available in the retail market in Australia.

Both sources tested for 4 nitrofurans metabolites; 3-amino-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ), 1-aminohydantoin (AHD) and semicarbazide (SEM). A dietary exposure assessment was conducted for each of these metabolites.

**Table 1: Nitrofurans residues in prawns from two analytical surveys**

Study	Total Number of samples analysed	Metabolite	Number of detections $\geq 1 \mu\text{g}/\text{kg}$	Actual detections ( $\mu\text{g}/\text{kg}$ )
AQIS	85	AOZ	4	3.2, 10.0, 34.7, 40.0
	85	AMOZ	0	
	85	AHD	0	
	85	SEM	1	8.9 (dried prawn only)
Qld Health	51	AOZ	6	1.1, 1.2, 1.3, 1.8, 2.0, 9.5
	50	AMOZ	1	
	51	AHD	0	
	51	SEM	0	

While the data from the Queensland survey are representative of the retail market in Australia on their own, the data from the two surveys were combined for the purposes of conducting the dietary exposure assessment. This was done to firstly increase the sample number, and secondly due to the likelihood that imported prawns that are not sampled coming into the country may have concentrations similar to those in the AQIS data and be available for purchase by consumers.

In order to derive a mean AOZ concentration for use in the dietary exposure assessment, a numerical number had to be assigned to ‘not detected’ results.

The limit of reporting (LOR) for both nitrofurans studies was  $1 \mu\text{g}/\text{kg}$ . The LOR is the lowest concentration of a chemical that can be detected and quantified, with an acceptable degree of certainty, using a specified laboratory method and/or item of laboratory equipment. Due to this, it may not be reasonable to assume that nitrofurans residues were not present in the food when the analytical result was less than the LOR. For this reason, where an analytical result was reported as below the LOR (or as ‘not detected’), the actual content could be anywhere between zero and the LOR. To allow for this uncertainty, the results of each ‘not detected’ food sample analysis were presented as a range, between which the likely concentration of nitrofurans would occur. The ‘lower bound’ of this range was calculated assuming that results reported as being less than the LOR were equal to zero. The ‘upper bound’ of this range, representing a conservative ‘worst-case’ estimate, was calculated assuming that all results reported as being below the LOR were present at the LOR ( $1 \mu\text{g}/\text{kg}$ ).

A lower bound mean and an upper bound mean concentration for AOZ were calculated using the combined data sets for use in the dietary exposure assessment. Concentrations used in the dietary exposure assessment are shown in **Table 2**.

Analytical data for cooked and raw prawns were combined for the exposure assessment. This is because the NNS does not specify in what form consumers purchased the prawns that they

consumed. The results for the dried prawns sampled by AQIS (n=3) were not included in the combined results because there was no consumption of dried prawns in the 1995 NNS.

There were two results for AOZ in the combined data set at 34 µg/kg and 40 µg/kg, with the next lowest level of AOZ being 9.5 µg/kg. As a result, the means for AOZ calculated, including the two higher values will result in the means being skewed upwards.

**Table 2: Lower bound and upper bound mean concentrations for AOZ used for conducting the dietary exposure assessment**

Metabolite	Total number of samples analysed	Lower Bound Mean (µg/kg)	Upper Bound Mean (µg/kg)
AOZ	134	0.80	1.72

### Assumptions in the dietary modelling

Assumptions made in the dietary modelling include:

- all the prawns contain nitrofurans residues at the specified concentrations;
- consumption of foods as recorded in the NNS represent current food consumption patterns;
- consumers always selected the prawns containing nitrofurans; and
- nitrofurans concentrations used are representative of the prawns available on the Australian market.

### Limitations of the dietary modelling

A limitation of estimating dietary exposure over a period of time associated with the dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

Consumption data based on 24-hours also overestimates usual daily consumption for occasionally consumed foods such as prawns.

### Estimated dietary exposures to AOZ

The estimated dietary exposures to AOZ are shown in Table 3.

**Table 3: Estimated dietary exposures to AOZ**

Metabolite	Estimated consumer mean dietary exposure		Estimated consumer 95 <sup>th</sup> percentile dietary exposure	
	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)	Lower bound µg/d *(µg/kg bw/d)	Upper bound µg/d *(µg/kg bw/d)
AOZ	0.060 (0.0009)	0.129 (0.0019)	0.200 (0.0030)	0.430 (0.0064)

\* Mean body weight for Australians from the 1995 NNS for respondents aged 2 years and above = 67kg

### **Summary of JECFA evaluation on furazolidone**

The Committee considered data from pharmacodynamic, pharmacokinetic, metabolism, acute and short-term toxicity, carcinogenicity, genotoxicity, reproductive, and teratogenicity studies as well as special studies on endocrine function and some clinical studies in humans (WHO, 1993).

A summary of the studies reviewed by JECFA and the conclusions from this report is below (WHO, 1993).

#### **Absorption, distribution, metabolism and excretion studies**

The distribution, excretion, and biotransformation of radiolabelled furazolidone were studied in rats, chickens, pigs, and humans. After oral administration, furazolidone was rapidly absorbed and the radioactivity was widely distributed, the highest levels being found in liver, kidney, fat, and muscle. It was rapidly metabolized and excreted predominantly in urine. In chicken and human urine, only trace amounts of unchanged furazolidone could be detected, and of the large number of metabolites found only some were identified.

In rat and pig urine, the common metabolite appeared to be the open chain cyanometabolite 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone. In pigs, a substantial portion of the metabolites was bound to macromolecules, and it appeared that approximately 15-40% of this bound fraction was bioavailable. However, the Committee questioned whether valid extraction procedures had been used to isolate these bound metabolites. The Committee noted that quantitative information on metabolites was lacking. However, the Committee questioned whether valid extraction procedures had been used to isolate these bound metabolites.

#### **Acute studies**

In acute oral toxicity studies in mice and rats furazolidone was slightly toxic; the LD<sub>50</sub> values were of the order of 1100 and 1500 mg/kg bw, respectively.

#### **Short-term studies**

Rats receiving furazolidone at doses in the range 0.5-50 mg/kg bw/day for 45 to 53 weeks showed hypertrophy of liver cells. Palpable mammary tumours and a decrease in body weight gain were observed at 50 mg/kg bw/day, although these studies were of poor quality and no detailed histopathology was available in one of the studies.

In dogs, dose levels of 5-25 mg/kg bw/day for 90 days duration or longer led to neurological symptoms and histological changes in the basal ganglia, together with testicular degeneration. It was noted that the available information was deficient by current standards and poorly reported.

A No Observed Effect Level (NOEL) could not be established from short-term studies performed with rats and dogs.

## **Reproduction and developmental studies**

Two three-generation reproduction studies were performed in rats. In one study rats were exposed to furazolidone at concentrations up to 100 mg/kg in feed. In the other study only female rats were treated with diets containing 500 mg/kg, but this concentration was gradually reduced to 250 mg/kg in order to avoid the observed growth depression. No effects on reproductive performance were observed in either study. The NOEL was equivalent to 12.5 mg/kg bw/day.

In a special study designed to evaluate the effects on the male reproductive system, rats exposed to a dietary furazolidone concentration equivalent to 33 mg/kg bw/day for 12 weeks exhibited testicular degeneration. At 16 mg/kg bw/day no effects were observed.

Neither embryotoxicity nor teratogenicity was observed in rabbits after oral administration of furazolidone at a dose of 30 mg/kg bw/day.

## **Long-term and carcinogenicity studies**

A carcinogenicity study was conducted in Swiss MBR/ICR mice, which received a diet containing concentrations of furazolidone equal to average daily doses of 12, 24, or 47 mg/kg bw/day for 13 months, followed by a control diet for 10 months. In the mid- and high-dose groups, a significant increase in the incidence of bronchial adenocarcinomas was observed in both sexes, and the incidence of lymphosarcomas was significantly increased in male mice.

In two long-term toxicity/carcinogenicity studies, furazolidone was administered in the diet to Fischer 344 and Sprague-Dawley rats at concentrations equivalent to daily doses of 12.5, 25, or 50 mg/kg bw/day for 20 months. In Fischer 344 rats, a significant increase in the incidence of mammary gland adenocarcinomas was observed in females in the high-dose group. In addition, an increase in the incidence of sebaceous gland adenomas and thyroid adenomas was observed in both sexes at 25 and 50 mg/kg bw/day and of basal cell epithelioma and carcinoma in males of the high-dose group. In the high-dose group of Sprague-Dawley rats, significantly increased incidences were reported for mammary adenocarcinomas in females and for neural astrocytomas in males. In both strains of rat, female animals showed a significant increase in the incidence of mammary neoplasms (benign and malignant combined) at all dose levels, but without a dose-response relationship.

## **Genotoxicity studies**

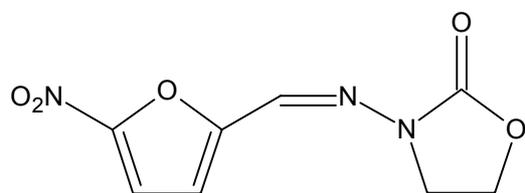
Furazolidone has been tested in a wide variety of genotoxicity studies. Positive findings were recorded in bacterial assays with and without metabolic activation, in the sex-linked recessive lethal test in *Drosophila melanogaster*, in a gene mutation assay with mammalian cells *in vitro*, in a sister chromatid exchange test, and in two DNA-repair tests. Positive as well as negative results were obtained in chromosome aberration assays with mammalian cells *in vitro*, and in tests for unscheduled DNA synthesis. One *in vivo* mouse micronucleus test was negative, while another gave equivocal results.

The majority of *in vitro* genotoxicity tests with postulated metabolites gave negative results; however, nitrofuraldehyde and urine from furazolidone-treated rats gave positive results. It was concluded that furazolidone was genotoxic *in vitro*.

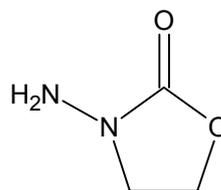
### **Other studies**

Several studies were performed on the endocrine effects of furazolidone. Furazolidone inhibited the conversion of progesterone into corticosterone in adrenal cells both *in vivo* and *in vitro*. It has been hypothesized that disturbances of steroidogenesis constituted the underlying mechanism for the increased incidence of tumours caused by furazolidone. The Committee noted that it was unlikely that such a mechanism could account for the increase in neural astrocytomas and uncommon skin tumours in rats. With respect to the occurrence of mammary tumours, no information was available on the effect of furazolidone on plasma progesterone concentrations and no consistent effects on plasma prolactin concentrations were observed. The Committee therefore concluded that no support had been provided for the hypothesized mechanism.

**APPENDIX 1**

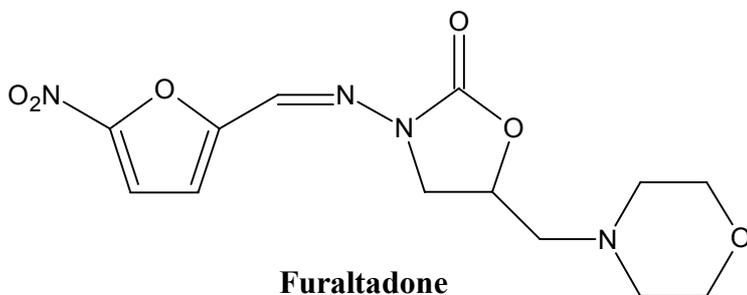


**Furazolidone**

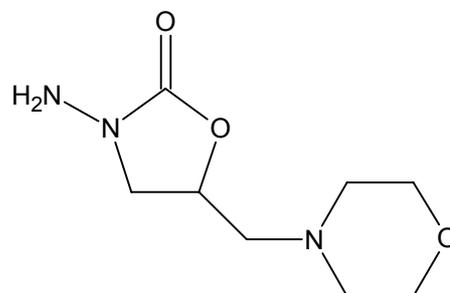


3-amino-2-oxazolidinone

**AOZ**

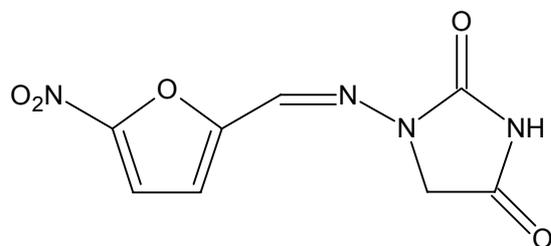


**Furaltadone**

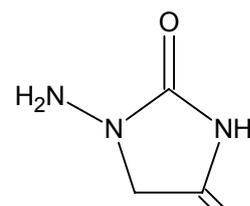


3-amino-5-morpholinomethyl-2-oxazolidinone

**AMOZ**

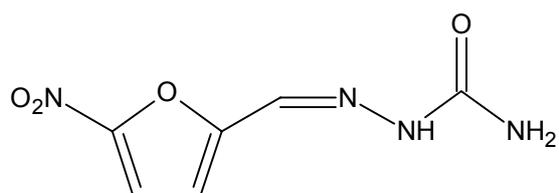


**Nitrofurantoin**

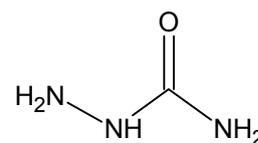


1-aminohydantoin

**AHD**



**Nitrofurazone**



Semicarbazide

**SEM**