
Australian Pesticides &
Veterinary Medicines Authority

# DRAFT REVIEW REPORT

# RECONSIDERATION OF THE REGISTRATION AND APPROVAL OF LABELS OF PRODUCTS CONTAINING DIMETRIDAZOLE

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**Australia**

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This draft review report for dimetridazole is published by the Australian Pesticides and Veterinary Medicines Authority. For further information about this review or the Veterinary Medicines Review Program, contact:

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# FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the *Agricultural and Veterinary Chemicals Code Act 1994* (Agvet Codes).

The APVMA can reconsider the approval of active constituents, the registration of chemical products or the approval of labels for containers of chemical products at any time. This is outlined in Part 2, Division 4 of the Agvet Codes.

The basis for a reconsideration of the registration and approvals for a chemical is whether the APVMA is satisfied that the requirements prescribed by the Agvet Codes for continued registration and approvals are being met. Reconsideration may be initiated when new research or evidence has raised concerns about the use or safety of a particular chemical, a product or its label.

The process for reconsideration includes a call for information from a variety of sources, a review of that information, and following public consultation, a decision about the future use of the chemical or product.

In undertaking reviews, the APVMA works in close cooperation with advisory agencies including the Office of Chemical Safety (OCS) within the Department of Health and Ageing, the Department of the Environment and Heritage, the National Occupational Health and Safety Commission (NOHSC), and State Departments of Agriculture as well as other expert advisors, as appropriate.

The APVMA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of review reports is part of that process.

The APVMA also makes these reports available to the regulatory agencies of other countries as part of bilateral agreements or as part of the Organisation for Economic Cooperation and Development (OECD) *ad hoc* exchange program. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the original data from the relevant applicant.

This report outlines the APVMA's review of products containing dimetridazole. The review's findings and proposed regulatory approach are based on information collected from a variety of sources, including data packages and information submitted by the registrants, information submitted by members of the public including users/industry groups and government organisations, and literature searches.

The review report containing the APVMA's preliminary assessments and the technical reports from its advisory agencies for all registrations and approvals relating to dimetridazole are available from the APVMA web site at http://www.apvma.gov.au/chemrev/chemrev.html.

## COMMENT FROM THE PUBLIC IS INVITED

The APVMA invites persons and organisations to submit their comments and suggestions on the review directly to the APVMA. These comments will assist the APVMA in preparing the final report.

In seeking comment, the APVMA emphasises the draft nature of this report and proposed regulatory approach, and expects that information obtained during the public comment period will result in further refinement and revision.

This report is derived from the toxicology, occupational health and safety, and residue assessments that were conducted by the APVMA's advisory agencies. The report describes the regulatory action that the APVMA proposes to take in relation to the continued registrations and label approvals of products that contain dimetridazole.

## PREPARING YOUR COMMENTS

You may comment on as many elements of the report as you wish.

When making comments, please:

- clearly identify the issue and clearly state your point of view;;
- give reasons for your comments, supporting them if possible with relevant information, and indicate the source of the information you have used; and
- suggest to the APVMA any alternative solution you may have for the issue.

Please structure your comments in point form referring each point to the relevant section in the review report. This will help the APVMA assemble and analyse all of the comments it receives.

Finally, please specify whether or not the APVMA may quote your comments in part or in full.

The closing date for comments is 7 **December 2004**.

Mail your comments to:

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# GLOSSARY

### Time

| | |
|---|---|
| **D** | day |
| **H** | hour |
| **Min** | minute |
| **Mo** | month |
| **Wk** | week |
| **s** | second |
| **yr** | year |

### Weight

| | |
|---|---|
| **Bw** | body weight |
| **G** | gram |
| **Kg** | kilogram |
| **µg** | microgram |
| **Mg** | milligram |
| **Ng** | nanogram |
| **Wt** | weight |

### Length

| | |
|---|---|
| **cm** | centimetre |
| **m** | metre |
| **µm** | micrometre |
| **mm** | millimetre |
| **nm** | nanometre |

### Dosing

| | |
|---|---|
| **Id** | intradermal |
| **Im** | intramuscular |
| **Inh** | inhalation |
| **Ip** | intraperitoneal |
| **Iv** | intravenous |
| **Po** | oral |
| **Sc** | subcutaneous |
| **mg/kg bw/d** | mg/kg body weight/day |

### Volume

| | |
|---|---|
| **L** | litre |
| **mL** | millilitre |
| **µL** | microlitre |

### Concentration

| | |
|---|---|
| **M** | Molar |
| **Ppb** | parts per billion |
| **Ppm** | parts per million |

### Chemistry

| | |
|---|---|
| **HPLC** | high pressure liquid chromatography |

### Terminology

| | |
|---|---|
| **ADI** | Acceptable Daily Intake |
| **Agvet Codes** | *Agricultural and Veterinary Chemicals Code Act 1994* |
| **ArfD** | Acute Reference Dose |
| **LOD** | Limit of Detection |
| **LOQ** | Limit of Quantitation |
| **LOEL** | Lowest Observed Effect Level |
| **MRL** | Maximum Residue Limit or Level |
| **NOEL** | No Observed Effect Level |
| **OHS** | Occupational Health and Safety |
| **SD** | Sprague Dawley |

### Organisations & publications

| | |
|---|---|
| **ACPH** | Advisory Committee on Pesticides and Health |
| **APVMA** | Australian Pesticides and Veterinary Medicines Authority (previously NRA) |
| **EMEA** | European Agency for the Evaluation of Medicinal Products |
| **FAO** | Food and Agriculture Organization (of the United Nations) |
| **FAISD** | First Aid Instructions and Safety Directions |
| **IARC** | International Agency for Research on Cancer |
| **JECFA** | FAO/WHO Joint Expert Committee on Food Additives |
| **NOHSC** | National Occupational Health and Safety Commission |

| | |
|---|---|
| **NRA** | National Registration Authority for Agricultural and Veterinary Chemicals (now APVMA) |
| **OCS** | Office of Chemical Safety |
| **PACC** | Australian Pesticide and Agricultural Chemicals Committee |
| **SUSDP** | Standard for the Uniform Scheduling of Drugs and Poisons |
| **US EPA** | United States Environmental Protection Agency |
| **USFDA** | United States Food and Drug Administration |
| **WHO** | World Health Organization |

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# EXECUTIVE SUMMARY

## Introduction

Products containing dimetridazole are currently approved in Australia for use in animals to treat and prevent diseases caused by certain infectious organisms. Dimetridazole is currently approved for the treatment and prevention of 'blackhead' in domestic and commercial poultry, and 'canker' in pigeons and caged birds. Blackhead is caused by a protozoan species, *Histomonas meleagridis.* Turkeys are particularly susceptible to *H. meleagridis* infection. Canker is caused by another protozoan species, *Trichomonas gallinae.* Dimetridazole products are also approved for the prevention of swine dysentery in pigs caused by the bacterial species, *Serpulina hyodysenteriae*. In addition, dimetridazole is used as a general anti-protozoal agent in pigs and poultry.

Registrations and label approvals for products containing dimetridazole are being reviewed as part of the APVMA's Review Program. The review was initiated in July 2002 because dimetridazole has been withdrawn from use in food-producing animals in several countries, primarily due to unresolved concerns regarding its potential carcinogenicity and uncertainty surrounding the longevity of residues in treated animals. Published reports have indicated that there is a potential for human exposure to residues of dimetridazole in food following the treatment of food-producing animals.

In this review, the APVMA, in collaboration with its advisory agencies, has completed an assessment of the data from the registrants, public submissions, scientific literature, archival holdings and reviews by overseas regulatory authorities.

## Public submissions

Eleven written submissions were received from State government departments, registrants and user groups. The concerns outlined in the submissions included:

- repeated observations that there is no suitable alternative chemical treatment available to control blackhead in poultry (particularly turkeys)
- the potential for an adverse economic impact on a range of business interests arising from this review, and
- the potential impact on human health, including worker safety particularly at the level of the end user, associated with the use of a chemical that is a possible carcinogen.

The concerns as outlined in the submissions received were considered in the review.

## Registrants' submissions

There were no new toxicological studies submitted by the registrants as a result of the data call-in. Many of the archived toxicological studies date back to the 1960s, and in some instances to the late 1950's and therefore do not conform to current test guidelines or levels of reporting.

## Toxicology assessment

The review reaffirmed the incompleteness of the toxicology database and finds that:

- no significant addition to the toxicology database has occurred since the previous evaluation to adequately address the issue of dimetridazole potential for carcinogenicity;
- data on metabolism of dimetridazole in turkeys and pigs are referenced in this review but no data on metabolism of dimetridazole in laboratory animals were submitted to this review. Such data are crucial in determining the relevance of animal models used in toxicity studies to address the potential toxicity of dimetridazole residues in humans;
- for a number of toxicology studies, only summary reports of studies were available for evaluation;
- experimental deficiencies in a number of toxicity studies, such as the use of only a small number of animals and/or monitoring of only a few study parameters, limited the studies' regulatory usefulness
- no observed effect levels (NOELs) for toxic endpoints could not reliably be set in many toxicity studies (including chronic, reproductive and developmental toxicity studies) because of absence of sufficient study details or deficiencies in study design;
- dimetridazole's potential to cause carcinogenicity and developmental effects in a second animal species was not assessed by OCS, however data from reproductive studies in pigs were considered in 1986 by the Australian Pesticide and Agricultural Chemicals Committee (PACC);
- in a recent 'comet' assay conducted in mammalian cells, dimetridazole showed evidence of genotoxic potential; and
- although dimetridazole did not show genotoxic effects in available *in vivo* studies, some related 5-nitroimidazoles (eg metronidazole, ronidazole) are genotoxic.

As a result of the toxicology assessment, this review finds that these deficiencies are significant and accepts the advice of the Office of Chemical Safety (OCS) that an acceptable daily intake (ADI) can no longer be supported to permit the continued use of dimetridazole in food producing animals. Nevertheless, it may be possible to allow the continued availability of dimetridazole products for the treatment of companion animals and birds.

## Occupational health and safety assessment

The data call-in notifications provided to registrants and other stakeholders primarily requested the provision of toxicological data in order to conduct a hazard assessment. However, the scope of the current review included the proviso that 'depending on the outcome of the toxicological assessment, an assessment of the occupational health and safety, food residue and trade issues may be required'.

Since no suitable toxicological data were submitted there was insufficient data on which to reliably establish effect levels to permit an occupational health and safety risk assessment to be modelled. Consequently, in the absence of suitable toxicological data a qualitative risk assessment was undertaken to identify suitable exposure control/mitigation measures for workers and safety directions for end users.

The review finds that the greatest risk of exposure to dimetridazole is likely to occur in feed milling processes, on-farm mixing and during transportation where dermal and inhalation (dust) exposures to existing products are possible. In order to reduce the risk from acute and repeated exposures, the use of personal protective equipment is recommended and new safety directions are placed on product labels. In addition, labels are required to warn users that dimetridazole is a possible genotoxic chemical.

### Residues assessment

The Chemistry and Residues Program of the APVMA (CRP) undertook a residues assessment based on existing available residues data for poultry. This assessment took into account that excess eggs from breeder chickens and breeder turkeys, and meat from culled breeder replacement pullets, may enter the human food chain. CRP investigated the feasibility of dimetridazole being permitted for use in poultry breeding stock. Since the OCS has recommended withdrawal of the existing dimetridazole ADI, all commodities from treated birds must have nil residues if they are to be made available for human consumption.

The review finds that from a residues perspective:

- the continued use of dimetridazole in breeder chickens and breeder turkeys is supported;
- the residue definition for dimetridazole should be amended to include the parent compound and its hydroxy metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole; suitable analytical methods are available in the published literature;
- the existing Maximum Residue Limits (MRLs) of dimetridazole for poultry meat and edible offal should be reduced from *0.005 mg/kg to *0.001 mg/kg, and an MRL of *0.001 mg/kg should be established for eggs;
- there is potential to reduce the meat and egg withholding periods from 28 days, but any such reduction would require industry stakeholders to provide additional residues data that meet contemporary standards, and
- product labels must also include an instruction that restrains users from administering dimetridazole to laying hens, broiler chickens and meat turkeys.

If the use of dimetridazole in breeding stock is to be supported:

(i) poultry producers must observe meat and egg withholding periods of 28-days, in order for residues in these commodities from breeder birds to be nil. At this residue level, these commodities can be considered suitable for human consumption, and

(ii) product labels are to include an instruction in the Directions for Use section that the products are for use in breeder chickens and breeder turkeys only

### Adequacy of label instructions

The review identified some deficiencies in current label instructions. The labels do not provide adequate instruction on the hazard potential of products containing dimetridazole, nor do they include any OHS exposure minimisation instructions. There are also inconsistencies between label withholding period statements. The

APVMA proposes to vary all labels to include new instructions to specify that the continued use of products containing dimetridazole is supportable in non-food-producing animals but not in animals from which edible commodities are produced for human consumption. It is also proposed to minimise worker and end-user exposure to dimetridazole during preparation and administration of dimetridazole-containing products to non-food-producing animals.

## Conclusions

This review concludes that since an ADI for dimetridazole can no longer be supported the registration of products currently used in food-producing animals will be cancelled.

The APVMA recognises that dimetridazole is an important tool in the management of blackhead in poultry and that there is no registered alternative chemical available to treat outbreaks of this disease. The APVMA is currently exploring options to permit the limited use of dimetridazole in breeder stock.

As a preliminary step, the APVMA initiated a residue assessment to ascertain the feasibility of such an approach. Based on the residues assessment, it appears that the APVMA may be able to support the continued use of dimetridazole in poultry breeding stock only. The residue assessment reports that the use of dimetridazole in breeder chickens and breeder turkeys while observing meat and egg withholding periods of 28 days is expected to result in 'nil residue' levels. This would not represent an undue risk to human health through dietary exposure. Furthermore, the use of dimetridazole with these limitations would not unduly prejudice Australia's export trade in poultry commodities. However, the APVMA must be satisfied that meat and eggs from treated breeder poultry can reliably be prevented from entering the food chain before the completion of the proposed withholding period.

Two regulatory options are proposed to support the ongoing use of dimetridazole: (i) use of the chemical be limited to companion animals only, with no use in any animal which may be consumed by humans, or (ii) use of dimetridazole in companion animals in addition to permitting limited use in non-food producing breeder poultry, breeder game birds, and breeder pigeons for squab production.

The review finds that ongoing use in companion animals and non-food producing breeder poultry, breeder game birds and breeder pigeons for squab production is supportable provided that labels are varied with the recommended label instructions and all restrictions are complied with. (Refer to Section 8 of this report for details of the new requirements.)

Assurance is required from industry, user groups and registrants that the recommended withholding periods for breeding stock, and restraints on using the chemical in non-breeding stock, will be observed. These limitations are also applicable to users of dimetridazole in squab pigeons and game birds. The APVMA intends to liaise with stakeholders on this issue to determine whether dimetridazole use in breeding stock can be managed by the industry, specifically the observance of 28-day withholding periods for meat and eggs.

## Summary of review recommendations

Based on the assessments in this review, the APVMA proposes that:

- the registration of dimetridazole products used only in food producing animals be cancelled;
- product labels be varied to specify that use of dimetridazole is restricted to non-food-producing animals and breeding stock of poultry;
- product labels be varied to provide instructions to users on ways to minimise exposure to dimetridazole;
- product labels be varied to include more detailed instructions for preparation and use; and
- the chemical dimetridazole be designated a 'possible genotoxic chemical'.

In addition, the ADI has been withdrawn by the OCS.

The APVMA is now seeking comment on suitable regulatory approaches to permit the ongoing use of dimetridazole in breeder chickens and breeder turkeys. If the APVMA can be satisfied that such use will not result in treated turkeys, breeder chickens or their eggs finding their way into the food chain prior to the 28-day withholding period the following changes are proposed:

- the residue definition for dimetridazole be amended
- the existing dimetridazole MRLs for poultry meat and edible offal be reduced and a new MRL be established for eggs, and
- a withholding period of 28 days be specified for meat and eggs.

# 1. INTRODUCTION

## 1.1. Regulatory status of dimetridazole in Australia

Dimetridazole is a derivative of 5-nitroimidazole. The systemic name is 1,2-dimethyl-5-nitro-1H-imidazole ($C_5H_7N_3O_2$). There are other 5-nitroimidazoles currently registered for use in humans and animals in Australia, generally for the treatment and prevention of protozoal infections. There are few alternative chemical treatments available for this purpose.

Products containing dimetridazole are currently registered for use in pigs, poultry, turkeys, game birds, pigeons and other caged birds. When this review commenced in July 2002, there were four registrants of seven veterinary products. The registration of one of these products has since not been renewed (refer to Appendix B for the list of registered products). The permitted uses for products containing dimetridazole are:

- treatment and prevention of blackhead (caused by *Histomonas meleagridis*) in game birds, poultry and other caged birds;
- treatment and prevention of swine dysentery (caused by *Serpulina hyodysenteriae*) in pigs; and
- as a general purpose antifungal and anti-protozoal in pigs and poultry.

Products containing dimetridazole may be applied as in-feed medication and/or by addition to drinking water.

No adverse effects associated with the use of any product containing dimetridazole have been reported to date to the APVMA's Adverse Experience Reporting Program.

## 1.2. Reasons for the review of dimetridazole

The decision to review products containing dimetridazole stems from concerns over human health.

The United States Environmental Protection Agency (US EPA) withdrew approvals for dimetridazole on the basis of its mutagenicity, and suspected carcinogenicity of its metabolites, but maintained some uses under veterinary prescription in non-food-producing animals.

The European Union (EU) also withdrew authorisation for the use of dimetridazole as a veterinary medicine over concerns about its potential carcinogenicity, whereas in Canada, the use of all 5-nitroimidazole chemicals in animals that produce food for human consumption has been prohibited due to a lack of toxicological information on bound residues of metabolites.

Moreover, the Joint Expert Committee on Food Additives (JECFA) reported that insufficient information is available to support the setting of an ADI or MRL for dimetridazole and later declared that dimetridazole is mutagenic *in vitro*. Details of these regulatory decisions are given in Section 8, Overseas Regulatory Status.

## 1.3. Scope of the review

In July 2002 the APVMA commenced the review of registrations and approvals relating to dimetridazole by releasing a scope document which made specific reference to concerns over the possible carcinogenicity of the chemical.

The basis for a review of the registration and approvals for a chemical is whether the APVMA is satisfied that the requirements prescribed by the Agvet Codes for continued registration and approval are being met. In the case of dimetridazole the relevant requirements were that the use of the product in accordance with the instructions for its use would not be likely to have an effect that is harmful to human beings and would not be an undue hazard to the safety of people exposed to it during its handling.

The July 2002 Review Scope Document stated that the review would be limited to the toxicological aspects of dimetridazole, but depending on the outcome of the toxicological assessment, an assessment of issues related to occupational health and safety, (OHS), food residue and trade may be required.

The requirements for product labels prescribed in the AgVet Codes are that the label contains adequate instructions. Such instructions include:

- the circumstances in which the product should be used;
- how the product should be used;
- the times when the product should be used;
- the frequency of the use of the product;
- the withholding period after the use of the product;
- the disposal of the product and its container;
- the safe handling of the product.

The July 2002 Review Scope Document canvasses an assessment of the adequacy of label instructions on current approved labels for products containing dimetridazole.

## 1.4. Regulatory options

In general there can be three possible outcomes to the review of the registration of products and their labels. Based on the information reviewed, the APVMA may:

- be satisfied that the products and their labels continue to meet the prescribed requirements for registration and approval and therefore confirm the registrations and approvals;
- be satisfied that the conditions to which the registration or approval is currently subject can be varied in such a way that the requirements for continued registration and approval will be complied with and therefore vary the conditions of registration or approval, or
- be not satisfied that the requirements for continued registration and approval continue to be met and suspend or cancel the registration and/or approvals.

# 2. TOXICOLOGY ASSESSMENT

The APVMA commissioned a report from the Office of Chemical Safety (OCS) in the Therapeutic Goods Administration (TGA). OCS re-evaluated previously submitted studies; evaluated newly submitted data; and considered some evaluations completed by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1990. The following paragraphs in this section are extracted from the OCS report.

## 2.1 Introduction

Dimetridazole belongs to a class of 5-nitroimidazoles, some of which are used to treat protozoal and bacterial (anaerobic) diseases in man and other animals. Structurally related compounds include metronidazole, tinidazole, nimorazole, ronidazole, ipronidazole, ornidazole and benznidazole.

In Australia, dimetridazole is one of two 5-nitroimidazoles registered for use in veterinary medicine. There are currently six registered products. Two products are pure dimetridazole powder (water insoluble), two products are water-soluble powders and the remaining two are premixed feed supplements. A table of registered products is at Appendix B.

The permitted uses of the products in Australia are treatment and prevention of:

- swine dysentery caused by *Serpulina hyodysenteriae*;
- blackhead in chickens, turkeys and game birds caused by *Histomonas meleagridis*;
- trichomoniasis (canker) in pigeons and caged birds caused by *Trichomonas gallinae.*

Products containing dimetridazole are also used as a general purpose antifungal and antiprotozoan in pigs and poultry.

Products containing dimetridazole may be applied as an in-feed medication and/or addition to drinking water. For disease prevention, labels recommend either continuous or regular feeding, while for treatment, dosing for between three and 14 days is generally recommended. Currently all products have a five-day withholding period for slaughter. The use of dimetridazole products in poultry producing eggs for human consumption is not permitted.

Dimetridazole is listed in Schedule 4 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). It has an Acceptable Daily Intake (ADI) of 0.002 mg/kg bw/d (set in 1986) based on a No Observed Effect Level (NOEL) of 3.8 mg/kg bw/d in a two-year rat dietary study and a 2,000-fold safety factor. The large safety factor was applied because the database on dimetridazole was incomplete.

Dimetridazole has received particular attention from regulatory authorities in the Australian Government Department of Health and Ageing, including OCS, because of concerns over its possible carcinogenic and mutagenic effects. In 1988, the United States announced the withdrawal of approval for dimetridazole over concerns relating to the potential carcinogenicity of residues. In response to these concerns, in 1990 the Australian Pesticide and Agricultural Chemicals Committee (PACC) evaluated an updated toxicology and residue package, and noted an increased incidence of benign mammary tumours in both sexes in a chronic dietary study in rats. The tumours occurred earlier in treated animals than in controls, with multiple tumours in affected

rats. The PACC stated that there appeared to be a clear dose above which the compound exerted its effect (a threshold level) and thus suggested that it was unlikely to be a genotoxic carcinogen. However, in order to reduce the possibility of human dietary exposure to dimetridazole, the committee recommended its use in poultry be limited to breeders. The committee also recommended that a Maximum Residue Level (MRL) be set at the limit of detection in pigs and poultry.

In 1990 JECFA evaluated the database for dimetridazole but could not establish an ADI for dimetridazole because of the absence of a carcinogenicity study in a second species.

In 1995 the European Union (EU) withdrew the authorisation of dimetridazole as a veterinary medicine over concerns regarding its carcinogenicity. In 1997 and 1999, Germany and Sweden respectively requested that the EU cancel the use of dimetridazole as a feed additive due to concerns over residues persisting beyond the withholding period and concern that it was a suspected genotoxic carcinogen. The EU's Scientific Committee on Animal Health in 2,000 concluded that dimetridazole was not a genotoxic carcinogen in mammals. This committee set a NOEL of 4.6 mg/kg bw/d and an ADI of 0.0046 mg/kg bw based on a 122-week rat study as well as a 1,000-fold safety factor. However, the EU and Canada, in 2001 and 2002 respectively, withdrew the authorisation of dimetridazole as a feed additive on the grounds that insufficient data had been submitted for a re-evaluation process.

## 2.2 Metabolism and toxicokinetics

The registrants of dimetridazole have not provided data to the APVMA on metabolism and toxicokinetics of dimetridazole in laboratory animals or in humans. In a rat study evaluated only by JECFA, it was noted that qualitatively similar metabolism was seen in rats and pigs. Metabolism via oxidation at the 2-methyl group and degradation of the nitroimidazole ring was common in these species (Heijbroek, 1976; cf. JECFA evaluation, 1990).

The 5-nitroimidazoles have been reported to undergo extensive metabolism. The biotransformation generally includes oxidation or reduction, with the ring structure remaining intact followed by scission of the ring to form an oxamic acid derivative (Koch and Goldman, 1979).

In turkeys (Law *et al.*, 1962; 1963), ~90% of a radiolabelled dose (32 mg/kg bw of $^{14}$C-dimetridazole, po) was excreted in urine, faeces and expired air within three days. No detectable levels (limit of detection = 0.03 μg/g tissue) were found in tissues of the turkeys three days after the last dose. Extensive metabolism was seen and the major metabolites included 1-methyl-5-nitroimidazol-2-yl methyl hydrogen sulphate (44% of the metabolites) and 1-methyl-5-nitroimidazol-2-yl methyl carboxylic acid (MNICA, 26%). 2-hydroxylmethyl-1-methyl-5-nitroimidazole (HMMNI, ~10%), a conjugated glucuronide and two unidentified non-nitro metabolites (<10%) were the minor metabolites.

In pigs (Unsworth, 1972; Mulcock and Unsworth, 1973a, b), total excretion after oral administration of $^{14}$C-dimetridazole (28 mg/kg/bw) in urine, faeces and expired air was 62% and 76% (of the administered dose) at two and seven days post-dose, respectively. Seven days after dimetridazole administration, tissue residues (up to 1 ppm) were found in several tissues, including the liver, kidney, spleen, fat and muscle. Only ~50% of the metabolites of dimetridazole were identified. The identified

metabolites (in urine or as residues in tissues) included HMMNI and MNICA, but there were no conjugated metabolites.

### 2.2.1 Acute studies

Dimetridazole exhibited low oral acute toxicity, with an $LD_{50}$ of 1,790 mg/kg bw in mice and 1600 mg/kg bw in rats. Clinical signs of toxicity in both rats and mice included sedation, and mortality was caused by respiratory arrest (Hood, 1962b). Dimetridazole appears to be a non-irritant on the skin or eyes (although in another study, dimetridazole was reported to be 'a very mild irritant'), but sufficient details were not provided in the studies conducted (Cosar, 1957; Hood, 1962c).

The acute iv $LD_{50}$ in mice was 290 mg/kg bw (Hood, 1962b). Rats treated with dimetridazole at 5,000, 10,000 and 20,000 $mg/m^3$ by inhalation for four hours showed signs of haemorrhage in lungs and cervical lymph nodes (Pullinger, 1976).

One product, Emtryl Soluble, containing 40% w/w dimetridazole showed an oral $LD_{50}$ of 1,700 and 2,500 mg/kg bw/d in mice and rats respectively. The iv $LD_{50}$ values were 60 and 70 mg/kg in mice and rats respectively (Hood, 1962b). Another product, Emtryl Premix, (22.5% dimetridazole) appears not to be a skin irritant (Hood, 1962c).

There were no other studies on the acute toxicity of dimetridazole or its products by the dermal route. The potential for skin sensitisation of dimetridazole or its products was also not fully investigated. Hood (1962c) observed no irritation by rabbits to Emtryl Soluble, but sufficient experimental details were not provided for OCS to assess this finding.

### 2.2.2 Short-term repeat-dose studies

Mice were given dimetridazole by gavage at doses of 100, 250, 500 or 1,000 mg/kg bw/d for five days (no control group). All animals in the highest dose group died. Parameters other than mortality were not recorded or reported (Cosar, 1957).

In a four-week study in rats given 50 or 100 mg/kg bw/d (by gavage) there were no treatment-related effects on clinical signs, haematology, clinical chemistry or histology (Cosar *et al.*, 1957).

In a dose-range finding study in dogs (1/sex/group, no control group), diets containing 0.36% or 1.08% of dimetridazole (~90 or 270 mg/kg bw/d) were fed to the animals for four weeks. Results submitted in summary form indicated that food consumption was markedly reduced in dogs of the 1.08% group when compared to the 0.36% group. The animals in the 1.08% group exhibited ataxia, which appeared to be more prominent in the hindquarters. Histological changes in the 1.08% group included petechial haemorrhages and nephrosis of kidney, haemorrhages of heart and spleen, central lobular cirrhosis and haemorrhages of liver. The lungs showed a proliferation of interstitial tissue, which reduced the air space area. Kidneys of animals from both groups showed cloudy swelling in the cells lining convoluted tubules and tubules comprising the medullary ray.

Mild atrophy of the seminiferous tubules with no mature spermatocytes present and moderate degeneration of spermatids were also noted in the testes of the male dog from the 0.36% group. The study (Salsbury Laboratories, 1962b; study summary was evaluated by JECFA, 1990) was not evaluated by the Australian Government Department of Health (since November 2001, the Department of Health and Ageing).

According to an earlier evaluation report by the Department of Health (1986), in dogs (2/dose) given dimetridazole at 50 or 100 mg/kg bw/d (presumably by the oral route), significant ataxia developed after 10–30 days of treatment, caused by micro-haemorrhages in the grey matter of the spinal chord. There was no damage to the neurones, which accounted for the regression of symptoms when the treatment had stopped. Haematology was normal.

### 2.2.3 Subchronic studies

Diets containing 0, 0.2, 0.4, 0.6, 0.8 or 1% of dimetridazole (approximately equivalent to 0, 200, 400, 600, 800 or 1,000 mg/kg bw/d) were fed to rats for 13 weeks. Results from the study were submitted only in summary form. Deaths (females only) were seen in the highest dose group. Clinical signs included ataxia, tilted head, anaemic appearance, excitation and convulsions in the animals which died. Data for bodyweight, food consumption or dimetridazole intake were not provided.

Males dosed with 0.8% and 1% dimetridazole had albumin in the urine. Histopathology revealed testicular atrophy or degeneration in all treated groups. Severe atrophy of seminiferous tubules and absence of spermatogenesis were noted in these groups. Other effects in the treated groups included a decrease in the number of primary follicles and an increase in the degeneration of the follicular epithelium in the ovaries (except in the 0.2% and 0.8% groups); gastritis (except in the 0.6% and 0.8% groups); and degenerative myocardial fibres and focal infiltrations of leucocytes in the heart (except in the 0.2% and 1% groups).

It is not clear whether the absence of a dose–response relationship for the changes in ovaries, stomach and heart were related to the small number of animals used for histological examination (3/sex/group). A NOEL could not be set for this study (Salsbury Laboratories, 1962a).

In a study in rats fed dimetridazole in the diet at 0, 50 or 100 mg/kg bw/d for three months, no treatment-related effects were reported on clinical signs, body weight gain or urinalysis, but no data were provided for the study. No abnormalities were seen at histopathological examination but the examination was conducted in only a small number of animals (up to 2 animals/sex/group). A NOEL could not be determined for this study because of the deficiencies in the study (Hood, 1962a).

In a study conducted in a small number of dogs (1/sex/group), dimetridazole was given by gavage at 12.5 or 50 mg/kg bw/d for three months. Clinical signs included ‘blood-shot eyes’ with mydriasis and increased excitability in all dogs. One dog at 50 mg/kg bw/d had contracted muscles (abdominal and hind legs, rigor of the tail). According to the study author, clinical chemistry and urinalysis did not reveal any abnormality in liver or renal function. In the 50 mg/kg bw/d group, chronic pyelonephritis, fatty changes in the liver, hypocellularity in the bone marrow, and abnormal thyroid colloid were seen. No NOEL could be reliably set from this study due to lack of study details and the small number of animals employed (Hood, 1962a).

Dogs were given dimetridazole at 16, 33, 66 or 132 mg/kg bw/d po for 13 weeks. In the 132 mg/kg bw/d group, one dog died and the remaining dogs were killed *in extremis*. Bodyweight gain and food consumption were decreased in the treated groups. Ataxia, anorexia, convulsions and opisthotonos were seen at 66 and 132 mg/kg bw/d. It is not clear whether haematology, clinical chemistry, organ weights,

gross pathology and histological examination were carried out. No NOEL was set from this study (Salsbury Laboratories 1962c; study was evaluated by JECFA, 1990).

Dogs (4/sex/group) were given dimetridazole at 0, 5, 10, 20, or 40 mg/kg bw/d po for 13 weeks. Except for one dog in the 40 mg/kg bw/d group which died while under anaesthesia for bone marrow biopsy, there were no deaths. There were no drug-related effects on clinical signs, bodyweight, ophthalmology, neurology, food consumption, urinalysis, haematology, biochemistry, organ weight or histopathology (Goyder *et al.*, 1974; study was evaluated by the Australian Department of Health in 1986 and by JECFA, 1990). It appears that 40 mg/kg bw/d was the NOEL for this study.

### 2.2.4 Chronic studies

Female rats were given dimetridazole in the diet at doses of 0 or 0.2 % (approximately equivalent to 0 or 130 mg/kg bw/d) for 46 weeks, followed by a control diet containing no dimetridazole for 20 weeks (Bryan, 1970). Mortality was higher in the treated group. There were no treatment-related effects on bodyweight. At week 65, there was a biologically significant increase in the number of rats with benign mammary tumours (fibroadenomas) in the treated group. The incidence of multiple mammary tumours was also increased in the treated group. A NOEL could not be set for this study.

Diets containing 0, 100, 400 or 2,000 ppm were fed to groups of rats for 122 weeks (0, 3.8, 15 or 78 mg/kg bw/d in males; 0, 4.6, 18 or 94 mg/kg bw/d in females) (Lowe *et al.*, 1976; study was evaluated by the Australian Department of Health in 1986; by JECFA in 1990). Mortality was increased in the 400 (females) and 2,000 (males and females) ppm groups. In the high dose group, bodyweight gains were ‘slightly’ lower in females, but food consumption was not affected by treatment. In this group, nodules appeared sooner and a higher incidence was noted when compared with the control and lower dose groups. In the 400 and 2,000 ppm groups, there was a dose-dependent increase in the incidence of benign tumours (adenoma, fibroadenoma, fibroma) of the mammary gland. An increase in tumour multiplicity was also observed in females of these groups. Malignant mammary tumours were not increased in any treated group. A NOEL of 100 ppm was set for this study.

Diets containing 0 or 10 ppm dimetridazole (0 or 0.45 mg/kg bw/d in males, 0 or 0.57 mg/kg bw/d in females) were fed to rats for 128 weeks (Lowe *et al.*, 1977). At the end of the study, survival rates were decreased in treated males (survival was 32% and 12% in control and treated groups respectively). Treatment with dimetridazole had no effect on clinical signs, bodyweights or food consumption. In the treated group, increased relative liver and ovarian weights were seen in males and females respectively (without treatment-related histological changes). In the liver, increased incidences of congestion in males, and bile duct hyperplasia and parenchymal cell degeneration in females, were seen in the treated group. The number of rats with malignant tumours was slightly increased in the treated group. In the treated group, the incidence of pituitary adenomas was decreased in males while the incidence of mammary tumours (malignant) was slightly increased in females.

The biological relevance of the small increase in the tumour incidence was not clear and statistically not significant. Compared with other strains (SD and Wistar), the strain used in the study (CFY) was found to have a higher incidence (~2 fold) of

spontaneous mammary tumours. A NOEL could not be set for this study (decreased survival and liver changes were observed at the only dose tested).

### 2.2.5 Reproductive toxicity study

Groups of weanling rats were maintained on a diet containing 0, 100 or 2,000 ppm dimetridazole (approximately 10 or 200 mg/kg bw/d) for ~80 days prior to the first mating and throughout the production of three generations. Dimetridazole markedly reduced weight gain and food intake of $F_0$ males in the 2,000 ppm dosing group. Except for increased mortality in '$F_{1b}$ offspring' (both treated groups), there were no other treatment-related effects. The mortality was attributed to the increased number of dams that ceased lactating (Dale 1975; study was evaluated by the Australian Department of Health in 1986; and by JECFA, 1990*)*. A NOEL was not set.

In four studies in which diets containing 200, 500, 1,500 or 2,000 ppm dimetridazole were fed to sows and boars during the reproduction cycle, no treatment-related effects on reproductive traits and litter were observed (Anderson 1972, 1973, 1974; Hutchings and Evans, 1973). Similarly, diets containing 125 or 250 ppm dimetridazole had no effects on reproduction in turkeys over two generations. At 500 pmm, a drop in fertility in one generation was observed when turkeys were fed from day-old (Lucas *et al*., 1967). PACC evaluated all five studies in 1986, but a NOEL was not set for each study.

### 2.2.6 Developmental toxicity study

Dimetridazole was administered by gavage to rabbits on gestation days 6-18 at 0, 30, 60 or 120 mg/kg bw/d. Dose-related maternal toxicity as evidenced by the reduction in food intake and bodyweight gain as well as abortions were noted in all treated groups. Post-implantation loss was increased at the high dose. There was a slight reduction in foetal and placental weight (dose levels not stated). A NOEL could not be set for this study (Tesh *et al.,* 1988; study was evaluated by JECFA only, 1990*)*.

## 2.3 Genotoxicity studies

### 2.3.1 Gene mutation in bacteria, fungi and cultured mammalian cells

In a few studies conducted in bacteria (mostly in *Salmonella typhimurium;* one study in *Escherichia coli*, *Citrobacter freundii* and *Klebsiella pneumoniae*), dimetridazole (≤282 μg/mL) in the presence and absence of metabolic activation was mutagenic (Voogd *et al.,* 1974; Wang *et al.,* 1975; Benazet & Cartier, 1977; Mourot, 1988). The mutagenic effect appeared to be dependent on bacterial nitroreductase activity since dimetridazole by itself (100 μg/mL), or its urinary metabolites collected from rats treated with dimetridazole (400 mg/kg bw po or iv), gave only negative results in mutagenicity tests conducted on a strain of *S. typhimurium* (TA 100 Frl) which did not have any nitroreductase activity (Thybaud *et al.,* 1988).

In yeast (*Saccharomyces cerevisiae* D4), dimetridazole (500 μg/mL, without metabolic activation) was mutagenic (Voogd *et al.,* 1980).

In mammalian cells (CHO-K1 cells), dimetridazole (≤7,500 μg/mL) did not induce forward mutation of the HGPRT gene in the presence or absence of metabolic

activation. Severe cytotoxicity was seen at ≥5,000 μg/mL (cytotoxicity seems to be increased in the presence of metabolic activation) (Cordier & Bonneau, 1985).

### 2.3.2 Chromosomal effect assays *in vitro*

Dimetridazole was devoid of clastogenic activity when tested on CHO-K1 cell line at 500–2,800 μg/mL without metabolic activation and at 10–820 μg/mL with metabolic activation (Fournier & Cordier, 1986a).

### 2.3.3 DNA damage and repair *in vitro*

Dimetridazole did not induce DNA repair in Chinese hamster lung fibroblasts (200 μg/mL; Richold *et al.*, 1981) and CHO cells (≤20 μg/mL, concentrations >20 μg/mL were cytotoxic; Ingham, 1981). However, positive results were observed in a comet assay in human lymphocytes (Re *et al.*, 1997).

A comet assay is a technique that is used for detecting damage to the DNA of animal and human cells following exposure to test substances *in vivo* or *in vitro*. In conjunction with other genotoxicity assays and *in vivo* studies in animals, the comet assay provides information on the potential for a substance to cause DNA damage, which may lead to cancer.

In the comet assay, dimetridazole (71–354 μM for dimetridazole; 10–50 μg/mL) and metronidazole (58–292 μM), showed evidence of DNA damage as revealed by a significant and concentration-dependent increase in tail moment (comet length x quantity of DNA in the tail) when aerobic conditions were used in the assay. The DNA damage caused by these compounds was reduced under anaerobic conditions and was abolished in the presence of metabolic activation. Antioxidants (8-hydroxyquinoline, 52–258 μM; vitamin C, 285–2850 μM; catalase, 83–249 U/mL; and superoxide dismutase, 36–144 U/mL) induced a concentration-related protective response against the DNA damage caused by dimetridazole and metronidazole (Re *et al.*, 1997).

### 2.3.4 Gene mutation *in vivo*

A dominant lethal mutation assay in mice gave negative results (dimetridazole at up to 1,000 mg/kg bw/d, route of administration not stated, Dale, 1975; Dale, 1977). In this assay, 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI at 75 and 750 mg/kg bw/d po), a metabolite of dimetridazole, also was negative (Dale, 1977).

In *Drosophila melanogaster* fruit flies, dimetridazole administered to adult males either in the diet at 0.7, 1.4 or 2.8 mM (toxic at 2.8 mM) or by 'injection' (0.2 μL) at 7.1 or 35 mM (toxic at 35 mM) did not show any evidence of sex-linked recessive lethality when treated males were mated with untreated female insects. When dimetridazole was fed to larvae at 0.7, 1.4, 2.8 or 4.4 mM (toxic at 2.8 and 4.4 mM), dimetridazole induced significant increases in lethal mutations in some experiments, but the results were not reproducible. Hence dimetridazole (as well as metronidazole) was concluded not to be mutagenic in this assay. Out of the nine 5-nitroimidazoles (including dimetridazole and metronidazole) tested in this assay, compounds such as ZK 26.173 and ZK 25.095 (moxnidazole) were 'clearly' mutagenic while nimorazole and ronidazole were 'marginally' mutagenic (Kramers, 1982).

### 2.3.5 Chromosomal effect assays *in vivo*

In two micronucleus assays in mice, dimetridazole administered at 305–915 mg/kg bw po or 220 mg/kg bw intraperitoneally did not significantly increase the incidence of micronucleated polychromatic red blood cells in the bone marrow (Oud *et al.*, 1979; Fournier & Cordier, 1986b).

### 2.3.6 DNA damage and repair *in vivo*

In rats, dimetridazole at 1,000 mg/kg bw/d did not increase the incidence of unscheduled DNA synthesis in hepatocytes (Melcion & Cordier, 1988).

## 2.4 Hazard assessment

### 2.4.1 Adequacy of the database

The toxicological database on dimetridazole is poor in its coverage and quality. Many of the studies date back to the 1960s, and in some instances to the late 1950s. These studies are now of limited regulatory value because of the limited range of measurements made and/or the level of detail in the reports. Data gaps include:

- the availability of only JECFA evaluation summaries, and not full study reports for some pivotal studies, including a carcinogenicity study in rats that was used previously to set the NOEL, reproductive and developmental toxicity studies (see Table 1);
- the absence of a carcinogenicity study in a second species;
- no data on metabolites in mammals; and
- the absence of dermal toxicity, inhalational toxicity, and skin sensitisation studies conducted with the active constituent dimetridazole or its products.

These deficiencies are serious and there does not appear to have been any attempt to address them.

### 2.4.2 Metabolism and toxicokinetics

The metabolism and toxicokinetics of dimetridazole are not well characterised in laboratory animals or in humans, and hence the assessment of dimetridazole has to rely on data available for other 5-nitroimidazole chemicals and on data available in target species (studies in turkeys and pigs). Although a rat study was cited in a submission to PACC (February 1987) and to the JECFA (1990), no data have been submitted to OCS. It appears from the limited information provided that in rats, absorption (oral) and excretion of dimetridazole are ‘quick’. Dimetridazole is also reported to be 'extensively metabolised', with the formation (oxidation of the 2-methyl group) successively of 2-hydroxymethyl-1-methyl-5-nitro-imidazole (HMMNI) and 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA), which was excreted in the urine. Such metabolites were also found in turkeys and pigs. According to the JECFA (1990), metabolism in rats is similar to that in pigs. However, the OCS could not verify this.

### 2.4.3 Acute toxicity

Dimetridazole exhibits low oral acute toxicity. Clinical signs of toxicity in both rats and mice were sedation for 24 hours (Hood, 1962b). Haemorrhage in lungs and cervical lymph nodes were seen in rats when dimetridazole at very high concentrations (5,000, 10,000 and 20,000 mg/$m^3$) was administered by inhalation (for 4 hours).

Although data on the skin and eye irritation studies were not adequate since details about individual animal scores were not provided, there was no evidence of skin or eye irritation. There were no studies to determine either the acute dermal toxicity or potential for skin sensitisation.

Studies on the products were limited. Emtryl Soluble (containing 40% dimetridazole) showed low oral toxicity in mice and rats and was not a skin irritant. Only a skin irritation study was conducted in rabbits with Emtryl Premix (containing 22.45% dimetridazole) and this study did not reveal any significant skin irritation.

### 2.4.4 Short-term repeat-dose studies

Studies conducted in mice, rats and dogs gave only limited information. Deficiencies in these studies included limited parameters measured, insufficient numbers of animals in the tests, and/or the absence of a control group for comparison.

A mouse study monitored only one parameter, ie mortality (the maximum non-lethal dose was 500 mg/kg bw/d, po) and dimetridazole treatment was given only for five days. There was no control group in the study.

In a four-week study in rats given 50 or 100 mg/kg bw/d (presumably oral), there were no treatment-related effects reported on clinical signs, haematology, clinical chemistry or histology. However, histological examination was conducted on only 1 or 2 animals/sex/group and hence a meaningful interpretation of the study results is difficult.

In two dog studies, the number of animals used was low (2/group), and there was no control group in one study. From the available data, dogs appear to be more sensitive with respect to central nervous system effects than mice or rats, since ataxia was seen in dogs at ≥50 mg/kg bw/d po. It is not clear whether the central nervous system effect was a 'direct' (pharmacological or physiological) effect of dimetridazole or caused by haemorrhage in the spinal cord. Haemorrhages were seen not only in the spinal cord but also in different organs (kidneys, heart, spleen and liver, at ~270 mg/kg bw/d po). Toxic effects in kidneys (nephrosis), liver (central lobular cirrhosis in addition to haemorrhages) and lungs (proliferation of interstitial tissue) were also seen in dogs at ~270 mg/kg bw/d po while testicular toxicity (mild atrophy of seminiferous tubules, no mature spermatocytes, degeneration of spermatids) was seen at a lower dose (~90 mg/kg bw/d).

Because of study deficiencies and toxic effects seen at the low dose used in these studies, a NOEL could not be determined for any of these studies.

### 2.4.5 Subchronic studies

Two studies have been conducted in rats, but neither of these studies could be used for setting a NOEL in rats. One study was only in a summary form, and toxic effects (in testes, ovaries, stomach and heart) were seen at all tested doses. In a second rat study (evaluated in 1986; data not available for re-evaluation) there were no significant concerns except that the number of animals used for histological examination was low (1 or 2/sex/group).

Three studies were conducted in dogs, but only three of the five available studies were evaluated by the PACC in 1986. However, none of the study reports are now available for re-evaluating the data. Central nervous system effects, including ataxia and convulsions, were seen in two studies at doses ≥50 mg/kg bw/d. In a study evaluated in 1986, the NOEL was 40 mg/kg bw/d, the highest dose in the study.

Overall, in only one dog study could a NOEL (40 mg/kg bw/d) be set.

### 2.4.6 Carcinogenicity studies

Three studies have been conducted in rats to address the potential carcinogenicity of dimetridazole. However, a major deficiency in the database is that there was no carcinogenicity study in a second species ie mouse.

Only one dose was used in two of the studies and hence a dose-response relationship could not be assessed in these studies. A third rat study used three doses (100, 400 and 2,000 ppm) and this study was evaluated by the Australian Department of Health in 1986. In this study, a NOEL of 100 ppm (3.8 mg/kg bw/d) was set, based on benign mammary tumors occurring at higher doses. However, the United States Food and Drug Administration (US FDA) did not accept this NOEL since 40%, 54%, 90 % and 56% of males and 22%, 32%, 14% and 14% of females in the 0, 100, 400 and 2,000 ppm groups respectively were not subjected to histological examination to detect the presence of mammary tumors.

Anther study conducted with a lower dose of dimetridazole (10 ppm) showed that there was no statistically significant increase in mammary tumors, but a problem in this study as well as in the previous study (in which three doses were used) was that the rat strain used (CFY) had a high background incidence of mammary tumors in females (~70% of animals had benign or malignant mammary tumors; Lowe *et al.*, 1977). Because of the high background incidence of mammary tumors in this strain (when compared to the incidences of 25% in SD and 36% in Wistar rat strains) it is unlikely that small treatment-related increases in mammary tumor incidences would be detected in this strain.

A study was conducted in SD rats but only one dose was used (0.2% in diet, 2,000 ppm). In this study, benign mammary tumors were increased significantly at the tested dose but the effects of lower doses in this strain are not known. Another drawback of the SD rat study was that only females were tested and hence carcinogenic effects, if any, in SD males are not known. However, in the CFY strain, incidences of mammary tumors were increased at both 400 and 2,000 ppm in males and hence mammary tumors in rats are not sex-specific.

Other than mammary tumors, there were no biologically significant increases in the incidences of other tumors in any of the three carcinogenicity studies conducted in rats.

Dimetridazole is a 5-nitroimidazole and several related 5-nitroimidazoles are known to be carcinogenic. Thus, the 5-nitroimidazoles – metronidazole, ipronidazole and ronidazole –have been reported to be carcinogenic (see Table 1 below). A common finding with all three compounds was that the compounds increased mammary tumors in rats (similar to dimetridazole) and lung tumors in mice. Based on structural similarities, dimetridazole may also increase lung tumors in mice, although this potential has not been tested. No tumors of any kind have been reported in humans treated with metronidazole. Metronidazole is normally used therapeutically for less than a month, although it may be used for up to three months in cases of Crohn's disease. However, humans are not usually exposed to metronidazole for a prolonged period. With respect to dimetridazole, prolonged human exposure is possible by eating meat containing dimetridazole and metabolite residues. While metronidazole has been taken during all stages of pregnancy with no apparent adverse effects, its use during the first trimester is not recommended.

**Table 1. Comparative toxicity of some 5-nitroimidazoles**

| Chemical | Genotoxicity | Carcinogenicity | Reproductive and developmental toxicity |
|---|---|---|---|
| Metronidazole[1] | Genotoxic *in vitro* and *in vivo*[1].<br><br>The Ames test was positive while several nonbacterial tests in animals were negative. In patients with Crohn's disease, metronidazole increased chromosomal abnormalities in circulating lymphocytes[2]. | Carcinogenic in mice (≥2 mg/kg bw/d, gavage) and rats (≥35 mg/kg bw/d, gavage).<br><br>Mammary tumours in rats; lymphomas and lung adenomas in mice[1]. | Reproductive toxicity in long-term studies in mice and rats: hypo-spermatogenesis; decreased prostate and testis weights[1].<br><br>In mice, increased incidences of dead and malformed fetuses at 15 mg/kg bw/d ip[1].<br><br>EMEA concluded that 'teratogenicity has not been sufficiently tested'[1]. |
| Tinidazole | Mutagenic in the Ames test[2]. | Animal carcinogenic studies are inadequate to exclude tumorigenic potential[2]. | Animal studies suggest that tinidazole may have teratogenic potential[2]. |
| Ipronidazole | Mutagenic in bacteria; inadequate study design in mammalian test systems[3]. | Increase in lung tumours in mice at 1,000 ppm (150 mg/kg bw/d), and in mammary tumours in rats at 2,000 ppm (110 mg/kg bw/d)[3]. | In a 3-generation study in rats, reduced growth in pups and dams at 2,000 ppm (100 mg/kg bw/d). No embryo-fetal toxicity in mice and rats at ≤10 and ≤100 mg/kg bw/d respectively[3]. |
| Ronidazole | Genotoxic in bacteria and in the sex-linked recessive lethal test in *Drosophila melanogaster*; weakly positive or negative in the bone-marrow cytogenetic assay in mice; negative in the micronucleus tests and a | Lung adenoma or carcinoma in mice at 20 mg/kg bw/d; benign mammary tumours in rats at 10 and 20 mg/kg bw/d[3]. | Testicular toxicity in chronic studies[3].<br>In a 3-generation study in rats, no adverse effects on reproduction except for decreased number of pups at 800 mg/kg bw/d. In two other studies in rats, decreased fetal weight at 100 mg/kg bw/d[3]. |

| Chemical | Genotoxicity | Carcinogenicity | Reproductive and developmental toxicity |
|---|---|---|---|
| | dominant lethal assay[3]. | | In mice and rabbits, at up to 200 and 30 mg/kg bw/d respectively, maternal toxicity at the high dose, but no significant teratogenicity[3]. |

[1]European Agency for the Evaluation of Medicinal Products (EMEA), July 1997
[2]MIMS, 2003
[3]JECFA, 1990.

The mechanism of dimetridazole-induced mammary tumors is not clear. As with other 5-nitroimidazoles, and as seen in target animal residue studies, dimetridazole undergoes (or is expected to undergo) oxidation at the 2-methyl group, leading to the formation of metabolites such as HMMNI, MNICA and their conjugates, reduction (metabolites such as nitroso- and hydroxylamino-compounds) and ring scission (acetamide is a possible metabolite). While acetamide is a carcinogen in rats, compounds with a nitro group (dimetridazole and some of its metabolites including HMMNI) have the potential to initiate neoplastic processes as a result of covalent binding to DNA (US FDA, 1986).

In the carcinogenicity studies, the relevance of the rat as a model to address the carcinogenicity potential of dimetridazole and its metabolites is not clear. This is because data on the metabolite profile (including quantitative data) of dimetridazole in rats have not been provided, and residues in target species have not been fully characterised, and hence it is not known whether the metabolites formed in rats are qualitatively and quantitatively similar to the residues formed in target animals.

Overall, there are significant data gaps in addressing the carcinogenic potential of dimetridazole. On the basis of available data, dimetridazole is clearly capable of inducing tumors (benign lung neoplasms) in rats, and judging from the effects of other 5-nitroimidazoles in mice, dimetridazole may also be carcinogenic in mice. Dimetridazole and several 5-nitroimidazoles are mutagenic in bacteria, and with new evidence (comet assay) showing that dimetridazole, like metronidazole, can cause DNA damage in mammalian cells, the main concern is that dimetridazole has the potential to be a 'genotoxic carcinogen'.

### 2.4.7 Genotoxicity studies

Dimetridazole is clearly mutagenic in a variety of *in vitro* bacterial assays including *S.typhimurium* (TA100, TA100-FR, TA1530, His G46, TA1531, TA1532, TA1534 (Lindmark & Muller, 1976; Rosenkranz *et al.*, 1976), *K. pneumonia* (Voogd *et al.*, 1979; Voogd *et al.*, 1974), *E.coli* (Voogd *et al.*, 1974) and *C. freundii* (Voogd *et al.*, 1974; Voogd *et al.*, 1979). Some of the studies (see below) were not assessed by the Australian Department of Health since a few full study reports which were submitted to JECFA for evaluation were not submitted to that Department.

The Australian Department of Health's evaluation reports in 1986 and 1987 did not contain any statement regarding mutagenic activity of dimetridazole in bacteria and it appears that no study reports on bacterial mutagenic activity were submitted to the Department in these years. Later, the Department noted that an Ames study in *S.*

*typhimurium* (Mourot, 1988) was evaluated by JECFA in 1990, but in spite of a request (February 1991) by the PACC to the sponsor (Rhone-Poulenc) to submit this study for evaluation, the study was never submitted. The mutagenic effect of dimetridazole in bacteria has been claimed to be due to the presence of nitroreductase enzyme in the bacterial cells, but no data have been submitted to the OCS in support of this claim (see below).

A concern is that structural analogues of dimetridazole, such as ipronidazole, ronidazole, metronidazole, 2-methyl-5-nitroimidazole, ornidazole and nimorazole, have also been shown to be mutagenic (see Table 1 above). According to the US FDA (1986), at least twenty 5-nitroimdazole chemicals have been found to be mutagenic to *S. typhimurium*. Metronidazole and ipronidazole are mutagenic in the same bacterial systems as dimetridazole, namely *S. typhimurium, E. coli, K. pneumoniae* and *C. freundii* (Mohn *et al.*, 1979; Voogd *et al.*, 1977, 1979; Voogd, 1981). The fact that all three compounds are mutagenic in the same test systems is especially noteworthy because ipronidazole and metronidazole have been shown to be mutagenic in mice (Anon, 1985; Cavaliers *et al*., 1983, 1984; Rustia & Shubik, 1979). Thus 5-nitroimidazoles, as a group, have the potential to induce mutagenic effects in bacteria.

Although dimetridazole is mutagenic in bacteria and also in some eukaryotic cells (*S. cervevisaie*), other tests conducted in mammalian cells (*in vitro*) and in insects (*D. melanogaster)* and mammals (mice, rats) indicated that dimetridazole was not genotoxic in these assay systems. The positive effect seen in bacteria and in *S. cerevesiae* was attributed to the presence of nitroreductase activity in these cells, since dimetridazole did not show any mutagenic activity in a bacterial strain deficient in this enzyme (*S. typhimurium* TA 100 Frl; Thybaud *et al.*, 1988). It is to be noted that this study (Thybaud *et al.*, 1988), as in the case of the Ames test (Mourot, 1988; see above), was evaluated by JECFA but not independently assessed by the Australian Department of Health. Moreover, two other studies in mammalian cells (evaluated by JECFA, 1990; unscheduled DNA synthesis in Chinese hamster lung fibroblasts, Richold *et al.*, 1981; *in vivo* study in rats on DNA damage and repair, Melcion & Cordier, 1988) were not submitted for evaluation in spite of a request (in 1991) by the PACC to the sponsor.

Most of the *in vitro* assays were conducted in the presence and absence of metabolic activation with S9 prepared from rat liver but it is not clear whether the residues found in target animals (metabolites such as HMMNI and MNICA, or their conjugates) were also formed in the genotoxic assays, in the presence of metabolic activation by S9. A metabolism study has been reported as having been conducted in rats (as per a submission to the PACC in February 1987; RMB Animal Health Ltd, May & Baker report RES/2498) but the study data do not appear to have ever been submitted. In this rat study, the metabolism was reported to be similar to that in the turkeys and pigs, but this information could not be verified because of the absence of data. Hence it is not clear whether the *in vitro* (with metabolic activation) and *in vivo* models used are relevant to address the genotoxic potential of dimetridazole metabolites.

The genotoxicity study by Thybaud *et al.* (1988; see above; data not submitted) appears to have addressed this issue at least partly since the study report (as evident from the study title) appears to discuss (or provide evidence for) the roles of liver S9 mix, bacterial nitroreductases and *in vitro* metabolism in the liver and the intestinal flora on the mutagenic activity of dimetridazole in the Ames test.

The metabolites HMMNI and MNICA (formed from HMMNI) are also suspected to be mutagenic. This potential was not investigated in bacterial assay systems which showed positive responses to the parent compound. In a dominant lethal mutation assay in mice, both dimetridazole and a metabolite 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) gave negative results. However, other metabolites of dimetridazole were not tested in this assay. Although adequate studies are not available to address the mutagenic potential, it is known that an analogous 2-hydroxymethyl metabolite of metronidazole is five to 10 times more mutagenic than the parent compound in *S. typhimurium* TA 1535 (US FDA, 1986). Hence, the mutagenic potential of the metabolites of dimetridazole cannot be ruled out.

The results of a comet assay conducted in 1997 (Re *et al.,* 1997) in human lymphocytes are of concern with respect to chromosomal damage. In this *in vitro* study, dimetridazole and metronidazole, in the absence of metabolic activation by S9, were found to cause significant and concentration-dependent DNA damage. Metabolic activation reduced the genotoxic damage and hence it is likely that dimetridazole *per se* (or its intracellular metabolites) is capable of causing genotoxicity. The magnitude of the genotoxic response was reduced by anaerobic conditions and abolished by antioxidants (8-hydroxyquinoline, vitamin C, catalase and superoxide dismutase). These results suggest that dimetridazole induces oxidative DNA damage, a mechanism that may be different to that operating under anaerobic conditions. Hence, dimetridazole may have the potential to induce genotoxicity in human cells, functioning in an aerobic environment.

Although dimetridazole did not show any effect in chromosomal effect assays (*in vitro* or *in vivo*), increases in the incidences of chromosomal aberrations have been reported in metronidazole-treated mammalian cells under aerobic conditions (Korbelik & Horvat, 1980). Some investigators have suggested that the genotoxicity of metronidazole may have been at least partly associated with the reduction of the nitro group of metronidazole by nitroreductase under anaerobic conditions (Korbelik & Horvat, 1980; Rosenkranz *et al.*, 1982; Rosenkranz & Mermelstein, 1983). A similar mechanism may be responsible for the mutagenic effects of dimetridazole in bacteria. However, in the comet assay (Re *et al.,* 1997), the magnitude of the genotoxic response was reduced by anaerobic conditions and abolished by the inclusion of antioxidants (8-hydroxyquinoline, vitamin C, catalase or superoxide dismutase). These results suggest that dimetridazole induces oxidative DNA damage, a mechanism that may be different from that operating under anaerobic conditions, thus suggesting dimetridazole may have the potential to induce genotoxicity in aerobic human cells. For these reasons, mutagenic effects of dimetridazole observed in bacterial assays cannot be dismissed as bacterial-specific effects which have no relevance to humans.

The reason for the absence of genotoxic effects in the *in vivo* studies is not clear. However, as pointed out before, until the metabolism of dimetridazole in the species used (*D. melanogaster*, mice and rats) is better characterised, the results of the *in vivo* studies (not only genotoxicity studies, but also other toxicity studies) should be interpreted with some caution.

The methodology used in an *in vivo* DNA damage and repair study (only one study; Melcion & Cordier, 1988) has been questioned. The EU's Scientific Committee on Animal Nutrition had noted that this study had not been performed to current OECD guideline 486 that was adopted in 1997. This guideline recommends an early (at two–

four hours post-dose) as well as a later sampling time point (12–16 hours), noting that some compounds such as dimethylnitrosamine would not have been detected as positive if only the later sampling time point was used. In the Melcion & Cordier study, the only sampling time was 15 hours. It is to be noted that this study was requested by PACC (1991), but the study was not submitted by the sponsor. Based on available data, the Scientific Committee on Animal Nutrition concluded that further data were needed to provide adequate assurance that the mutagenic activity seen *in vitro* was not expressed *in vivo*.

Overall, there are data deficiencies in trying to assess the genotoxic potential of dimetridazole. The available information indicates that the potential of dimetridazole to be genotoxic cannot be ruled out.

### 2.4.8 Reproductive and developmental toxicity

In the literature, metronidazole, ipronidazole and ronidazole have been reported to adversely affect reproductive performance and developmental toxicity (see Table 1). In chronic studies, metronidazole and ronidazole have been previously shown to cause testicular toxicity.

Studies on dimetridazole revealed testicular toxicity (histological changes) at high doses (≥200 mg/kg bw/d) in subchronic studies in rats. However, male fertility was not affected in a three-generation reproduction study in rats and hence testicular effects are unlikely to be a significant concern. In this three-generation reproduction study, increased pup mortality was seen with dimetridazole treatment in only one generation ($F_{1b}$ offspring), an effect which appeared to be due to decreased lactation in dams. It is not clear whether dimetridazole has a direct effect on lactation or whether lactation was affected by some other mechanism eg the presence of mammary tumours. Since only one generation was affected, it was not completely clear whether the changes seen were treatment-related. Because of the uncertainties, a NOEL could not be set for this study.

The potential for developmental effects was only partially investigated. Only one species (rabbits) was used to study the effect of dimetridazole on developmental toxicity but the study (Tesh *et al.*, 1988) was never submitted to the Australian Department of Health. In the evaluation by JECFA (dimetridazole at 30, 60, 120 mg/kg bw/d po), post-implantation loss (120 mg/kg bw/d) a 'slight' reduction in fetal and placental weights (dose levels not stated) were seen in the treated groups, but the effects were associated with maternal toxicity (dose-dependent reduction in food intake and bodyweight gain; abortion) in all treated groups. A NOEL was not established for maternal toxicity, and in the absence of detailed study results, a NOEL could not be set for developmental effects either.

### 2.4.9 Concerns evident in toxicity studies

The following concerns were evident in the toxicity studies and in the database submission:

- crucial studies submitted to overseas agencies were not submitted in Australia;
- there is new evidence that dimetridazole is genotoxic in mammalian cells (comet assay); hence there is a potential that dimetridazole could be a genotoxic carcinogen;

- data on metabolism and toxicokinetics of dimetridazole in turkeys and pigs are referenced in this review but no data on metabolism and toxicokinetics of dimetridazole in laboratory mammals were submitted to this review. Such data are crucial to understand the relevance of animal models used in toxicity studies in determining the human toxicity of residues formed in target animals;
- there are no data in a second species for carcinogenicity;
- data in a second species for developmental toxicity were not available to OCS for assessment although data for dimetridazole from pig studies were considered in 1986 by PACC; and
- NOELs could not be set in many studies because of deficiencies in the studies; the previously used NOEL of 3.8 mg/kg bw/d in a rat carcinogenicity study could not be reliably considered as the overall NOEL to set an ADI because of the above concerns.

## 2.5 Dose levels relevant for risk assessment

The OCS used a two-year dietary study in rats (Lowe *et al.*, 1976) to set the previous NOEL in 1986. In this study, the NOEL was found to be 3.8 mg/kg bw/d based on benign mammary tumours (Lowest Observed Effect Level (LOEL): 15.1 mg/kg bw/d). The maximum safety factor of 2,000 was used to derive an ADI of 0.002 mg/kg bw/d since the database was incomplete.

In the previous evaluation, a NOEL of 40 mg/kg bw/d was established in a 13-week oral dog study (see Table 2 below). A NOEL could not be set for many other studies. The current OCS toxicological evaluation of dimetridazole has found the available database insufficient to determine a reliable overall NOEL. Hence, the OCS could not determine an overall NOEL and ADI nor affirm the existing ADI that was set in 1986. Furthermore, the available data are insufficient to set an ARfD.

**Table 2. Establishment of a NOEL**

| Species | Study type | NOEL (mg/kg bw/d) | LOEL (mg/kg bw/d) | Effect | Reference |
|---|---|---|---|---|---|
| Rat | 2-year dietary | 3.8 | 15.1 | Benign mammary tumours | Lowe *et al.*, 1976 |
| Dog | 13-week oral | 40 | 40 mg/kg bw/d was the highest dose used. | - | Goyder *et al.*, 1974 |
| An overall NOEL could not be determined because of data deficiencies. | | | | | |

### 2.5.1 Diet

#### *2.5.1.1 Australian Total Diet Survey and Australian National Residue Survey*

The 1999–2,000 Australian National Residue Survey, conducted under the auspices of the then Department of Agriculture, Fisheries and Forestry, Australia, monitored dimetridazole residues in pigs but not poultry. No residues were found in pig muscle tissue (limit of reporting [LOR] = 0.001 mg/kg) from 148 analyses. Similar results were noted in the 1997 Australian National Residue Survey. Dimetridazole has not

been included in the current or previous Australian Total Diet Surveys (formerly Market Basket Surveys).

#### *2.5.1.2 Acute and chronic dietary intake*

Acute and chronic dietary intake calculations are generally performed by the APVMA and Food Standards Australia New Zealand.

### 2.5.2 Water

Based on its registered veterinary uses, dimetridazole residues should not be found in water or water catchment areas. Therefore, no health level for dimetridazole in drinking water has been set.

### 2.5.3 Non-dietary exposure consideration

This consideration was not evaluated in the toxicology assessment. Refer to Section 4, Occupational Health and Safety Assessment.

## 2.6 Consideration of public health standards

### 2.6.1 Approval status

A list of registered products and their approved uses is presented in Appendix B.

### 2.6.2 NOEL, ADI and ARfD

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health. It is calculated by dividing the overall NOEL from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans, intra-species variation, the completeness of the toxicological database, and the nature of the potential toxicologically significant effects.

The existing ADI for dimetridazole, set by the OCS in 1986, is 0.002 mg/kg bw/d. It was based on a NOEL of 3.8 mg/kg bw/d from a two-year chronic dietary study in rats and the incidence of benign mammary tumours at the next highest dose using a 2,000-fold safety factor due to poor data supporting registration. The OCS' current evaluation indicated that there are significant data gaps, and new concerns (regarding genotoxic potential) and hence a NOEL or ADI/ARfD could not be set using current available data. Therefore, the OCS cannot confirm the previously set NOEL or ADI.

### 2.6.3 Poison scheduling

Dimetridazole is currently listed under Schedule 4 (Prescription Animal Remedy) of the SUSDP.

### 2.6.4 First aid instructions and safety directions

Since dimetridazole is recommended for use in companion animals and birds, the current entry in the First Aid Instructions and Safety Handbook for first aid

instructions (ie '*a*': If poisoning occurs, contact a doctor or poisons information centre) should be retained.

At present, there are two products approved for use in companion animals and birds: Emtryl Soluble Dimetridazole Soluble Powder (containing 40% dimetridazole, w/w) and CCD Dimetridazole (Water Soluble Powder) (containing 100% dimetridazole, w/w). Currently there are no safety directions for dimetridazole products. No safety directions are required for the product CCD Dimetridazole (Water Soluble Powder) which contains only the active constituent. However, Emtryl Soluble Dimetridazole Soluble Powder contains calcium lignosulfonate, which is reported to cause contact allergy at or above 2%. Hence, the following statements are recommended for the product label:

**Safety directions**

| **Dimetridazole containing calcium lignosulfonate** | |
|---|---|
| Repeated exposure may cause allergic disorders | 180 |
| Wash hands after use | 351 |

# 3. IMPLICATIONS OF THE TOXICOLOGY ASSESSMENT

## 3.1 Uses of dimetridazole

As a result of the toxicological database not containing sufficient data to support the existing ADI, the OCS will delete this entry from the ADI List (http://www.tga.health.gov.au/docs/pdf/adi.pdf). Consequently, uses of dimetridazole in food-producing species will not be supported, while uses in pigeons, caged birds and game birds will be retained providing that commodities from these species are not used for human consumption. Limited use of dimetridazole in replacement pullets of breeder chickens and breeder turkeys may continue, but would need to be supported from a residue perspective.

## 3.2 Residues

The residue implications include:

- changes may be required to the existing MRL entries for dimetridazole in poultry meat or offal and in pig meat or offal. There is no MRL entry in the MRL Standard for dimetridazole in eggs;
- there is need to ensure that the withholding periods are suitable as there should be no residues of dimetridazole in food intended for human consumption; and
- changes may be required to the residue definition of dimetridazole to account for metabolites metabolism cited in paragraph 2.2.

## 3.3 Trade

There are no trade implications associated with the recommendations detailed in this report as there should be no residues of dimetridazole in commodities exported from Australia.

## 3.4 Occupational health and safety (OHS)

From an OHS perspective, if the use of dimetridazole is restricted to non-food-producing species as a regulatory outcome, the risk to users and workers from exposure to dimetridazole should be assessed and mitigated.

The Australian Material Safety Data Sheet for dimetridazole should be updated to include information consistent with the findings of this review.

These implications have led to subsequent OHS and residues assessments, which are discussed in the next sections.

# 4. OCCUPATIONAL HEALTH AND SAFETY (OHS) ASSESSMENT

While the review focused primarily on toxicology issues, some of the findings are relevant to OHS. In reviewing the available information, it was recognised that data were not available to enable the identified concerns for worker safety to be comprehensively assessed. There were no exposure data available to estimate exposures, or suitable default methods available to assess risk to workers or domestic users. Therefore, a qualitative risk assessment was undertaken rather than a full OHS risk assessment that is based on margins of exposure. The OHS section of OCS conducted this assessment.

## 4.1 Hazard overview

### 4.1.1 Toxicity and hazard classification of dimetridazole and its products

From the toxicology assessment of available acute studies, the OHS assessment noted the toxicity profile of dimetridazole and the unavailability of data relating to the acute toxicity of dimetridazole or its products by the dermal or inhalation route, and the potential for skin sensitisation.

The OHS assessment also noted the difference in toxicity of products containing 40% or 22.5% w/w dimetridazole. One registered product contains calcium lignosulfonate (CAS No. 8061-52-7) at a concentration that is likely to cause contact allergy.

Based on the median oral $LD_{50}$ from acute toxicity studies in mice and rats, and its genotoxic potential, dimetridazole is classified as being hazardous. Therefore, the following risk phrases (and cut-off concentrations) apply in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2002 draft).

R22 Harmful if swallowed

R68 Possible risk of irreversible effects (Mutagen category 3)

| | | |
|---|---|---|
| Concentration cut-off: | greater than or equal to 25% | R68, R22 |
| | greater than or equal to 1% but less than 25% | R68 |

As the concentration of dimetridazole in the registered products ranges from 20% to 100%, all six products are also classified as hazardous.

## 4.2 Use profile and occupational exposure assessment

Two use scenarios have been identified for the six registered products. In the first scenario end-users add the product to feed in mixers, or to the drinking water, at the same time that they incorporate vitamin and mineral premixes.

In the second scenario, feed millers prepare medicated feed in feed mills where mixing occurs. Details of these operations are not available. The use profile of each registered product is listed in Table 3 below.

**Table 3. Use profiles for dimetridazole products**

| Product name and pack size | DMZ* concentration (percentage of DMZ) | Species indicated | Label instruction (maximum and minimum percentage of DMZ) |
|---|---|---|---|
| CCD DMZ 225 Premix (Dimetridazole) (20 kg) | 225 g/kg (22%) | Turkeys<br>Chickens<br>Pigs | For treatment: 2.2 kg/tonne feed for 7–14 days (0.06% maximum)<br><br>For prevention: 340g/tonne feed) fed continuously (0.01% minimum) |
| CCD Dimetridazole (Water Soluble Powder) (25 kg) | 1,000 g/kg (100%) | Turkeys<br>Pigs<br>Caged birds | For prevention (turkeys and pigs): 120 g/1,000L of water (0.01% minimum)<br><br>For prevention (caged birds): 250–600 mg/L water (0.06% maximum) |
| Dimetridazole FG (20 kg) | 200 g/kg (20%) | Pigs | 1,000 g/tonne of finished feed (0.02%) |
| Emtryl Premix for Feed Medication (20 kg) | 200 g/kg (20%) | Pigs<br>Poultry | 2.5 kg of the product per tonne of finished feed (0.05% maximum)<br>375 g/tonne (0.01% minimum) |
| Emtryl Soluble Dimetridazole Soluble Powder (25 kg) | 400 g/kg (40%) | Pigs<br>Turkeys<br>Chickens<br>Pigeons | 25 g/40 L water (0.02% maximum)<br>25 g/120L water (0.01% minimum) |
| Bronson and Jacobs Dimetridazole (DMZ) Oral (25 kg) | 1,000 g/kg (100%) | Turkeys<br>Pigs | 500 g/tonne finished feed (0.05% maximum)<br>75 g/tonne finished feed (0.01% minimum) |

DMZ = dimetridazole

Workers who are considered at potential risk of exposure by inhalation and/or dermal contact have been identified and grouped as those who are:

- involved in the manufacture/formulation (suppliers) of dimetridazole products;
- transporting the products;
- preparing the medicated feed in feed mills;
- using the product.

Persons at greatest risk of exposure are likely to be those involved in feed milling processes, where dermal and inhalation (dust) exposure to dimetridazole products is possible. Workers who are exposed to mixes are unlikely to be significantly exposed to dimetridazole due to low concentrations of 0.01% to 0.06% of dimetridazole in the mixes. End users may also come into dermal contact with dimetridazole during preparation of the final mix.

Should the ADI be withdrawn and the registered products no longer used in food-producing animals, it would be difficult to estimate the quantity of dimetridazole that will be used for the remaining label uses. There is a possibility that with this limitation on use, manufacture/formulation of dimetridazole products in Australia will

be significantly reduced; therefore, feed-milling operations may no longer be required.

The OHS assessment has determined that Good Manufacturing Practices and Hazard and Operability Studies could adequately control worker exposure during manufacture and formulation.

## 4.3 Occupational risk assessment

This assessment is qualitative in nature since it is not based on margins of exposure.

### 4.3.1 Risks associated with manufacture/formulation and transportation of dimetridazole products

Of the six registered dimetridazole products, five are manufactured in Australia. It is generally accepted that APVMA-registered manufacturers work under conditions of Good Manufacturing Practice, which includes attention to OHS by means of plant Hazard and Operability Studies. Several 5-nitromidazole analogues, which possess similar toxicological profile, are manufactured in Australia without reports of adverse effects, such as increased incidence of cancer in workers involved in manufacturing. Risks during manufacture are therefore likely to be low.

Risks during transportation of dimetridazole products will be minimal as all the products are sealed in bags. Packaging requirements are regulated under the Department of Transport's Australian Code for the Transport of Dangerous Goods by Road and Rail, which also addresses appropriate emergency procedures when packaging is breached.

### 4.3.2 Risks associated with exposure during end-use including workers preparing the medicated feed in feed mills

Data on dermal and inhalation toxicity, and eye and skin irritant studies in animals, are not available to characterise risks associated with the use of dimetridazole products. Risks will be highest during preparation of the feed in feed mills and on farms. During on-farm handling and mixing of products containing 20–100% dimetridazole, end users may be exposed via airborne dust. Once the feed is prepared for use, risks are likely to be minimal as the prepared feed will contain 0.01 to 0.06% of dimetridazole which is below the NOHSC concentration cut-off value of 1% for occupational genotoxicity hazard. Risks will be further reduced by any reduction in quantities handled, as a result of decreased usage.

## 4.4 OHS review outcomes

Exposure data would be required in order to characterise any risk to workers potentially exposed to dimetridazole and dimetridazole products. However, it is recognised that since the quantities of dimetridazole formulated and used in Australia are likely to be dramatically reduced as a consequence of the ADI withdrawal, it is unlikely that such data will become available.

Due to the lack of available worker exposure data and uncertainties over the NOEL for dimetridazole, the OHS assessment has attempted to identify exposure control/mitigation measures based on the available information. Safety directions and additional control measures that will mitigate potential acute and long-term

occupational risks have been identified should the APVMA decide to support the continued registration of dimetridazole products for non-food-producing animals such as caged birds and pigeons.

## 4.5 Conclusions

The conclusions in the OHS assessment address:

- the requirement for further information to characterise risks to workers
- evaluation of label requirements, and
- the requirement for additional OHS control measures under the NOHSC National Model Regulations for the Control of Hazardous Substances.

### 4.5.1 Requirement for further information

Information is required on the manufacture, formulation, milling and end use of each registered dimetridazole product. If continued use of dimetridazole in food-producing animals is not supported, information is still required on remaining uses of dimetridazole products in game birds and caged birds. Details to be provided include a projection of quantities of products to be used; number of workers involved in manufacturing and formulation; number of feed millers; and exposure data where available. Detailed use pattern information on all the registered products would also be needed.

### 4.5.2 Labelling requirements

A number of inadequacies have been identified on current labels for dimetridazole products with respect to instructions relating to OHS. As a result, label recommendations are made to reduce current acute and chronic OHS risks.

*Warning statement*

The following warning should be included on all approved labels for dimetridazole:

**Dimetridazole is a possible genotoxic chemical**.

*Safety directions*

In addition, all approved products are required to include the following safety directions:

**When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge).**

**After each day's use wash contaminated clothing, gloves and respirator and if rubber wash with detergent and warm water.**

These safety directions are considered sufficient to significantly reduce potential risks from both acute and repeated exposure to dimetridazole products.

*Reference to Material Safety Data Sheet*

Each approved label for dimetridazole should refer to a Material Safety Data Sheet for additional information.

### 4.5.3 Formulation change

The main concern for workers has been identified as potential inhalation of dust that contains dimetridazole from mixing and milling operations. In this regard OCS (OHS) considers that a change in formulation to reduce potential exposure to product dusts would significantly reduce risks to workers.

Without taking into account issues of feasibility, OCS (OHS) recommends that product suppliers consider reformulation of the products into water-soluble sachets. OCS (OHS) notes that two products intended for use in caged birds and pigeons are currently administered in drinking water and therefore this should not be an issue with regard to treatment efficacy.

Should products be reformulated so that potential inhalation of product powder/dust becomes negligible during normal work practices, the requirement for wearing a respirator when opening the container or preparing the mix could be cancelled.

### 4.5.4 Additional control measures under NOHSC Model Regulations for Control of Workplace Hazardous Substances (NOHSC 1994a)

The following OHS control measures outlined in paragraphs 4.5.4.1 and 4.5.4.2 are integral to the safe use of dimetridazole. Compliance with labeling instructions alone may not provide sufficient risk mitigation. These additional recommendations are made under the NOHSC Model Regulations for Control of Workplace Hazardous Substances under which all pesticide manufacturers and users should operate. The intention is for the relevant agencies in all States and Territories to adopt and enforce these recommendations. Product registrants, users and OHS agencies are expected to be aware of these additional risk mitigation measures.

#### *4.5.4.1 Availability of labels for dimetridazole*

Labels prepared in accordance with NOHSC Labelling Code Guidelines (NOHSC 1994b) should be available to all workers involved in the manufacture or formulation of dimetridazole products. The labels should state the hazard classification for dimetridazole, including its genotoxicity potential.

#### *4.5.4.2 Material Safety Data Sheets for dimetridazole products*

Material Safety Data Sheets should be prepared in accordance with NOHSC Code of Practice for the Preparation of Material Safety Data Sheets (NOHSC 1994c). A material safety data sheet should be available to all users of the products. It should reflect the hazard classification outlined in paragraph 4.1.1 and potential genotoxicity of dimetridazole products. Personal protective equipment recommended for handling all dimetridazole products should include a chemical resistant overall buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator with dust cartridge.

# 5. RESIDUES ASSESSMENT

## 5.1 Introduction

This assessment is based on existing available residues data for poultry, because no new residues data were provided. In conducting this assessment, the APVMA's Chemistry and Residues Program has taken into account the fact that excess fertilised eggs and meat from culled replacement pullets/breeders and spent hens may be sold or processed for human consumption. The Chemistry and Residues Program further noted the OCS recommendation that the use of dimetridazole in food-producing animals be discontinued.

In the absence of an ADI and/or an ARfD for dimetridazole, no residues of dimetridazole would be acceptable in edible commodities. Therefore, the residue assessment was conducted to determine whether available residue data are supportive of a regulatory approach whereby the continued use of dimetridazole in squab pigeons and game birds is permitted, but the use in poultry is restricted to breeders. Commercial layers and broilers are excluded from treatment.

## 5.2 Toxicological information

The APVMA's Chemistry and Residues Program considered OCS' findings that there are insufficient toxicology data to set an ADI or ARfD, and that the existing dimetridazole ADI will be withdrawn. Existing health standards for dimetridazole are provided in paragraphs 2.5 and 2.6.2 in this report.

## 5.3 Current dimetridazole approved use patterns in poultry

Of the five registered products containing dimetridazole that are approved for use in poultry, the pioneer products Emtryl Premix for Feed Medication (P38037) and Emtryl Soluble Dimetridazole Soluble Powder (P38038) were first considered for registration in 1986. Residues data were submitted with the applications. The remaining three products, Bronson and Jacobs Dimetridazole (DMZ) Oral (P50141), CCD Dimetridazole (Water Soluble Powder) (P50743) and CCD DMZ Premix (Dimetridazole) (P52812) are generic products, and no additional residues data were provided with the applications for registration.

The currently approved use patterns for dimetridazole in poultry are listed in Table 4.

**Table 4. Currently approved use patterns for dimetridazole in poultry**

| Animal | Purpose | Maximum dose rate | Critical comments |
|---|---|---|---|
| Turkeys, chickens | For treatment of blackhead (Histomoniasis) | Medicated drinking water<br>Provide medicated water as the only source of drinking water for 12 days. Treat birds with 500 mg DMZ*/L water for the first 3–6 days, then treat with 250 mg DMZ/L water for the balance of the 12 days. | Medication for birds should be withdrawn during periods of high environmental temperature, where thirst may increase, or where dehydration may occur. |
| Turkeys | For the treatment and/or prevention of blackhead | Medicated feed<br>*Treatment:* Provide in-feed at a rate of 500 mg DMZ/kg feed for 7–14 days.<br><br>*Prevention:* Provide in-feed continuously at a rate of 125 mg DMZ/kg feed. | |
| Chickens | For the treatment and/or prevention of blackhead | Medicated feed<br>*Treatment:* Provide in-feed at a rate of 500 mg DMZ/kg feed for 7–14 days then continue medication at the preventative level.<br><br>*Prevention:* Provide in-feed continuously at a rate of 75 mg DMZ/kg feed. | |

*DMZ = dimetridazole

Withholding periods on existing labels are:

MEAT: DO NOT ADMINISTER later than 5 days before slaughter for human consumption.

EGGS: DO NOT USE in birds which are producing, or may in the future produce, eggs or egg products for human consumption.

### 5.3.1 Residues history of dimetridazole in poultry

Between July 1971 and August 1989, the Pesticides and Agricultural Chemicals Standing Committee (PACSC) held a series of meetings to consider the residues of dimetridazole in meat of poultry, turkeys and pigeons. The PACSC concluded that there was a special use for dimetridazole in breeders, and that the drug should be restricted to use in turkeys, pigeons and poultry breeders. The assumption was that eggs from breeder birds would not be made available for human consumption, and so an egg MRL was never established.

## 5.4 Data and analytical methodology considerations

### 5.4.1 Metabolism of dimetridazole in rats

In a study (Heijbroek 1976) evaluated only by JECFA, it was reported that when $^{14}$C-dimetridazole is orally administered to rats, it is rapidly absorbed and excreted. The parent drug is extensively metabolised, with the successive formation of 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) and 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA), which is excreted in the urine. The pattern of dimetridazole metabolism in rats was reported to be similar to that observed in pigs and turkeys.

### 5.4.2 Metabolism of dimetridazole in pigs

Dimetridazole is rapidly absorbed in pigs following a single oral dose. About 40–60% of the administered dose is excreted within 24 hours of treatment (~75% in urine and 25 % in faeces). By seven days post-treatment, excretion has increased marginally to 40–70% (50–75% in urine and 25–50% in faeces). In expired air samples, about 3.3% and 4% of the administered dose are recovered within 24 hours and seven days respectively of dosing.

In one study (JECFA 1989), analysis of the urinary residues excreted within 24 hours of dosing showed that 50–65% of the residues were made up of 5-nitroimidazole compounds, that is, the parent dimetridazole and its metabolites containing intact nitro groups. In a second study, chromatographic examination of urine from pigs collected during the first eight hours after dosing revealed the presence of dimetridazole (0.2% of total urinary activity), HMMNI (0.7%) and MNICA (18.7%). Conjugation of metabolites was not found to be a major pathway in pigs. Much of the urinary radioactivity was found to be associated with simple, naturally occurring compounds, such as amino acids. The composition of the faecal residues has not been determined.

When pigs were treated with a single oral dose of 19–37 mg $^{14}$C-dimetridazole/kg bw and sacrificed at seven days post-treatment, the levels of radioactive residues in edible pig tissues were 0.15 to 0.5 mg equiv/kg in muscle; 0.20 to 0.56 mg equiv/kg in fat; 0.6 to 1.1 mg equiv/kg in liver and 0.4 to 1.1 mg equiv/kg in kidney (Unsworth 1972). These levels were significant.

In a second study (JECFA 1989), pigs were sacrificed at six hours after treatment with a single oral dose of 29.8 mg $^{14}$C-dimetridazole/kg bw. HMMNI was found to be the major identifiable component in the residues of muscle and kidney. The results are tabulated in Table 5 below.

**Table 5. Identity and quantity of dimetridazole residues in edible pig tissues at six hours post treatment**

| Compound | Dimetridazole residues | | | | | |
|---|---|---|---|---|---|---|
| | Muscle | | Kidney | | Liver | |
| | mg/kg | % of TRRs* | mg/kg | % of TRRs | mg/kg | % of TRRs |
| Dimetridazole | 0.04 | 0.5 | 0.18 | 0.5 | 0.01 | 0.07 |
| HMMNI | 3.56 | 40.2 | 10.31 | 25.7 | 0.09 | 0.5 |
| MNICA | 1.33 | 13.8 | 1.55 | 3.6 | ND | ND |
| **Total** | **4.93** | **54.5** | **12.04** | **29.8** | **0.10** | **0.57** |

*TRR = total radioactive residues

In a similar experiment (JECFA 1989) in which a pig was dosed with 16.6 mg $^{14}$C-dimetridazole/kg bw, the only metabolite detectable in muscle 17 hours after dosing was HMMNI, which represented approximately 10% of the tissue radioactivity, equivalent to ~0.04 mg/kg.

### 5.4.3 Metabolism of dimetridazole in turkeys

Dimetridazole is also rapidly absorbed and excreted from turkeys following a single oral dose (Law et al 1962). 30–80% of the administered dose was recovered from the excreta of treated turkeys within 24 hours of treatment, and 60–100% was recovered within 72 hours. About 90% of the residues in excreta were water-soluble (extracted with water and 0.1 M hydrochloric acid). Less than 2% of the administered dose was recovered in expired air samples.

The water-soluble residue components that were identified in turkey excreta using a series of paper chromatographic systems were:

- a sulfate conjugate of dimetridazole, 1-methyl-5-nitroimidazol-2-ylmethyl hydrogen sulfate (44.4% of the total excreted drug);
- 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA) (25.8%);
- 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) (9.4%);
- unchanged dimetridazole (3.4%);
- a glucuronide conjugate of a 5-nitroimidazole compound (8.8%). Positive identification of this metabolite was not possible in the absence of appropriate reference compounds; and
- a non-nitro compound (6.2%), which was postulated to be a derivative of 1,2-dimethyl-5-aminoimidazole.

A further non-nitro compound (comprising 4.7% of the total excreted drug) was detected on autoradiograms, but did not absorb ultra-violet (UV) radiation, which is indicative of a degraded imidazole ring.

As stated before, the metabolite profile for dimetridazole residues in edible turkey tissues (and eggs) has not been fully explained. In one study, where turkeys were treated with a single oral dose of $^{14}$C-dimetridazole, and sacrificed at 72 hours post-treatment, the levels of radioactive residues in edible tissues (kidney, liver, muscle and skin) were reported to be below the method LOQs of less than 0.03mg/kg to less than 0.05 mg/kg. However, these results are not conclusive, since the assay methods were not validated, and they employed a benzene extraction step, which is unlikely to efficiently extract the water-soluble residue components from tissues.

Nevertheless, the major biotransformation products of other drugs belonging to the 5-nitroimidazole class, for example dimetridazole analogues such as metronidazole and ipronidazole, are qualitatively the same in all species studied. Therefore, it is concluded that the metabolism of dimetridazole in turkeys is likely to be extensive, and would follow the same metabolic pathways as dimetridazole in pigs.

Overall, the metabolism of dimetridazole is believed to include:

- oxidation at the 2-methyl group, to give the hydroxymethyl (HMMNI) and carboxylic acid (MNICA) metabolites. It is noted that binding of HMMNI to proteins is likely to result in persistent residues in edible tissues of treated animals;

- reduction at the 5-nitro group to give an amino compound, which would undergo rapid degradation. The nitro group supposedly reduces stepwise via nitroso and hydroxylamino intermediates to the amine. These intermediates, particularly the hydroxylamino, can covalently bind to protein or DNA. This binding of chemical electrophiles to cellular macromolecules is accepted as a mechanism by which chemical carcinogens initiate the neoplastic process; and
- fission of the 5-nitroimidazole ring. It is noted that one postulated product of this breakdown process is acetamide, a known chemical carcinogen.

### 5.4.4 Analytical methods

Details of the polarographic method used to determine dimetridazole residues in edible poultry tissues and eggs during residue trials were provided as part of the residue reports. In principle, the polarographic method would measure the levels of dimetridazole and any metabolites containing the nitro group (including HMMNI and MNICA), since the method involves reduction of the nitro moiety to an amino group. However, the method is dependent on the efficiency with which these analytes are extracted from tissues using benzene.

For poultry tissues, samples were homogenised with water or buffer solution, then extracted with benzene. The benzene extract was partitioned against an acidified aqueous solution. The aqueous phase, containing the dimetridazole analytes, was (sometimes) cleaned up with carbon tetrachloride to remove fats and other compounds that interfere with polarography or was mixed with an aqueous alkaline solution. Subsequently, the aqueous extract was saturated with borax, then thoroughly deoxygenated with oxygen-free nitrogen. Finally, a polarogram was recorded (-0.2 V to -0.9 V versus a Ag/AgCl or Hg electrode), and the residue levels were determined using an external standard calibration curve.

In the case of eggs, the benzene extraction procedure was not used. Instead, the yolk and albumen from each egg was separated, and aliquots of each fraction were saturated with borax, deoxygenated, and polarograms were recorded.

Validation data for the polarographic method were provided for dimetridazole (linearity $r^2$ of 0.9990 over the concentration range 0.05 to 5.0 mg/L; recovery range of 68 to 130% from samples fortified with 0.1 to 2.5 mg/kg dimetridazole), but not for the metabolites. The method LOQ for tissues and eggs was reported to be 0.1 mg/kg.

Owing to the relatively high water solubility of HMMNI and MNICA, it is contended that the levels of these analytes in the benzene extract are likely to be low. Therefore, the polarographic method is not considered to be adequate for regulatory purposes, because it has not been fully validated and its LOQ of 0.1 mg/kg is not sufficiently sensitive.

A search of the published literature revealed that there are a number of analytical methods available to measure dimetridazole residues in edible tissues. Details of some of these methods are provided in the following table.

**Table 6. Validated analytical methods for the determination of dimetridazole residues**

| Method description | Reference | Analytes | Samples | LOQ/LOD (mg/kg) | Comments |
|---|---|---|---|---|---|
| *HPLC/UV detection | 1 | DMZ** HMMNI | Eggs | LOQ 0.005–0.010 | |
| | 18 | DMZ | Poultry meat<br>Pig meat | LOD 0.005<br>LOQ 0.010 | |
| | 47 | DMZ<br>HMMNI | Poultry meat<br>Eggs | LOD 0.0005 | Method used as part of the UK surveillance program. |
| | 50 | DMZ<br>HMMNI | Turkey meat<br>Pig meat | LOQ 0.001 | Method used as part of the US surveillance program. |
| Liquid chromatography | 48 | DMZ<br>HMMNI | Pig tissues | LOD 0.001–0.002 | |
| HPLC with thermospray MS/MS*** | 32 | DMZ<br>HMMNI | Turkey tissues | LOQ 0.002 | Method used to confirm identity of analytes detected with HPLC/UV method. |
| GC/NICI/MS | 50 | DMZ<br>HMMNI | Turkey meat<br>Pig meat | LOQ 0.001 | Method used by the US to confirm identity of analytes detected with the HPLC/UV method. |
| HPLC with atmospheric pressure chemical ionisation MS | 47 | DMZ<br>HMMNI | Poultry meat<br>Eggs | LOD 0.0001–0.0005 | Method used by the UK to confirm identity of analytes detected with the HPLC/UV method. |
| HPLC with electrochemical detection | 7 | DMZ<br>HMMNI | Pig tissues | LOQ 100–200 pg/sample | |
| Gas chromatography | 41 | HMMNI | Pig muscle | LOD 0.001<br>LOQ 0.002 | |
| High performance TLC**** with fluorescence detection | 19 | DMZ<br>HMMNI | Pig meat<br>Poultry meat | LOQ 0.005–0.010 | |

*HPLC = high pressure liquid chromatography

**DMZ = dimetridazole

***MS = mass spectrometry

****TLC = thin layer chromatography

Both the United States and the United Kingdom employ an HPLC method with UV detection for monitoring and compliance purposes. The methods determine the levels of dimetridazole and its hydroxy metabolite (HMMNI) in edible tissues and eggs, and have an LOQ of 0.001 mg/kg.

### 5.4.5 Residue definition

The existing Australian residue definition for dimetridazole in Table 3 of the MRL Standard consists of the parent compound only. This residue definition is not considered to be appropriate, since the results from metabolism studies have shown that the hydroxy metabolite (HMMNI) is the major component of residues in edible tissues from treated animals. Suitable analytical methods are available to measure residues of dimetridazole and HMMNI in edible tissues and eggs down to concentrations of 0.001 mg/kg.

As a result, the Chemistry and Residues Program recommends that the residue definition for dimetridazole be amended to:

> **The sum of dimetridazole and its hydroxy metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole, expressed as dimetridazole.**

### 5.4.6 Residue trials

Table 1 in the MRL Standard lists the MRLs for dimetridazole in meat and edible offal of poultry as 0.005 mg/kg. In the absence of an ADI or ARfD for dimetridazole, all commodities from treated animals must have nil residues if they are to be made available for human consumption.

The APVMA has been advised that edible commodities from breeder birds in the chicken and turkey industries may enter the human food chain under the following circumstances:

- when the supply of fertilised eggs exceeds the hatchery requirements, excess eggs may be sold for human consumption;
- when replacement pullets/breeders are culled, meat from these birds may be sold or processed for human consumption; and
- when breeder birds have reached the end of their economic lifespan (‘spent hens’), meat from these birds may be sold or processed for human consumption.

In order to assess the residues aspects associated with the use of dimetridazole on broiler breeders and turkey breeders, the available residues data for poultry tissues and eggs were reviewed.

### 5.4.7 Dimetridazole residues in eggs

A single residues trial (Amis *et al* 1964) in which laying hens were orally dosed with 125, 250 or 500 mg dimetridazole/kg feed for three consecutive weeks was evaluated. Eggs were collected for up to six days following withdrawal of the medicated feed, and residues were determined directly in the albumen and yolk fractions using the polarographic method described in paragraph 5.4.4. The residues results have been corrected to reflect the maximum 1× label rate of 500 mg dimetridazole/kg feed (see Table 7).

**Table 7. Dimetridazole residues in eggs — corrected to the maximum 1× label rate (500 mg dimetridazole /kg feed)**

| Sampling time (days after last treatment) | Parameter | Dimetridazole residues (mg/kg) | | |
|---|---|---|---|---|
| | | Albumen | Yolk | Whole egg (minus shell) |
| 0 | Range | 4.0 – 8.4 | 1.2 – 7.8 | 3.2 – 6.7 |
| | Mean ± SD | 5.6 ± 1.2 | 4.5 ± 1.8 | 5.1 ± 1.0 |
| | *Upper 95% CL | 7.9 | 8.0 | 7.1 |
| | N | 14 | 14 | 14 |
| 1 | Range | 3.2 – 7.3 | 1.6 – 9.2 | 2.4 – 6.9 |
| | Mean ± SD | 4.8 ± 1.1 | 4.2 ± 1.7 | 4.6 ± 1.2 |
| | *Upper 95% CL | 7.0 | 7.6 | 6.8 |
| | n | 22 | 22 | 21 |
| 2 | Range | 0.2 – 0.9 | 0.8 – 4.6 | 0.6 – 2.0 |
| | Mean ± SD | 0.6 ± 0.2 | 1.4 ± 1.0 | 0.9 ± 0.4 |
| | *Upper 95% CL | 1.1 | 3.4 | 1.7 |
| | n | 13 | 13 | 13 |
| 3 | Range | 0.2 – 0.4 | 0.3 – 0.5 | 0.2 – 0.4 |
| | Mean ± SD | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 |
| | *Upper 95% CL | 0.4 | 0.5 | 0.5 |
| | n | 7 | 7 | 7 |
| 4 | #Range | <0.1 – 0.8 | <0.1 – 0.4 | <0.1 – 0.6 |
| | Mean ± SD | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| | *Upper 95% CL | 0.6 | 0.2 | 0.4 |
| | n | 19 | 19 | 19 |
| 5 | #Range | <0.1 – 0.1 | <0.1 – 0.1 | <0.1 – 0.1 |
| | Mean ± SD | <0.1 ± <0.1 | <0.1 ± <0.1 | <0.1 ± <0.1 |
| | *Upper 95% CL | 0.1 | 0.1 | 0.1 |
| | n | 8 | 8 | 8 |
| 6 | #Range | <0.1 | <0.1 | <0.1 |
| | Mean ± SD | <0.1 ± 0 | <0.1 ± 0 | <0.1 ± 0 |
| | *Upper 95% CL | <0.1 | <0.1 | <0.1 |
| | n | 6 | 6 | 6 |

*Upper 95% confidence limit
# Values recorded as <0.1 mg/kg were taken as 0.05 mg/kg for statistical purposes.

Since the polarographic method used to measure dimetridazole residues in eggs did not incorporate a benzene extraction procedure and the egg yolk and albumen fractions were assayed directly, the residues results are likely to have quantified the levels of all dimetridazole residue components containing an intact nitroimidazole ring. As such, the results do not precisely address the proposed residue definition for dimetridazole of parent dimetridazole plus the hydroxy metabolite, HMMNI. In fact, the results are likely to over-estimate of the residue levels that would be detected using an appropriate regulatory method. Nevertheless, the results from the egg trial provide useful information on the residue decline profile in eggs, and may be extrapolated to estimate when a 'nil residues' situation is reached.

The highest residues were observed in eggs collected immediately following the cessation of treatment or within one day of drug withdrawal. The maximum reported residue levels were 8.4 mg/kg in albumen, 9.2 mg/kg in egg yolk, and 6.9 mg/kg in whole egg (minus shell). At no time did the highest residues occur in the albumen and yolk of a single egg, which explains why the maximum residue observed in whole egg is less than that observed for both the albumen and yolk fractions.

Total dimetridazole residues in eggs declined rapidly, with eggs produced on the second day of drug withdrawal reported to have maximum levels of 0.9 mg/kg in albumen, 4.6 mg/kg in yolk and 2.0 mg/kg in whole egg. Residues in yolk, albumen and whole egg were reported to be below the LOQ of 0.1 mg/kg within six days of drug withdrawal.

The length of the withdrawal period required for egg residues to become zero may be estimated by:

- determination of the upper 95% confidence limits at each sampling time, and extrapolation of the regression lines for the decline data, out to 'zero' residues; and
- calculation of the number of half-life periods required for the residues in eggs to decline to 'zero', and multiplying this figure by the half-life estimate for dimetridazole residues in eggs.

When calculating the withdrawal period estimates, the 'nil residue' level was initially defined as the LOD of the most sensitive analytical method that can be used to quantify dimetridazole residues in eggs and tissues. This value is 0.0005 mg/kg.

Statistical analysis of the egg residue data reveals that dimetridazole residues in albumen are expected to decline (with 95% confidence) to less than 0.0005 mg/kg within nine days of the cessation of treatment. Similarly, residues in yolk are expected to be less than 0.0005 mg/kg within 11 days. Thus, dimetridazole residues in eggs are likely to be 'nil' within 14 days of drug withdrawal.

Dimetridazole residues in albumen have an estimated half-life of about 0.5 days, and residues in yolk have an apparent half-life of about 0.7 days. Thus, if a conservative half-life of one day is used, and the maximum dimetridazole residue in whole eggs is taken to be 10 mg/kg, then residues will have declined to less than 0.0005 mg/kg within 15 half-lives (equivalent to 15 days).

The estimated 14-day period for egg residues to decline to non-detectable is consistent with the physiological time frame for egg development. Egg follicles develop rapidly in about the last 10–14 days before ovulation, increasing in size from about 1 gram to about 20 grams. During this development phase, drug residues are deposited in concentric rings as the yolk is laid down. Therefore, yolks in eggs that are laid 14 days after the cessation of treatment are unlikely to have been rapidly developing during the treatment phase.

However, the APVMA has noted that the egg withdrawal period estimates are based on a limited data set of a single residue study that was conducted using an unvalidated analytical method, and that the method has a relatively high LOQ that necessitates extensive extrapolation. Therefore, on a risk assessment basis, the APVMA has considered it appropriate to apply a safety factor to the egg withdrawal period estimates, and set the egg withholding period at 28 days.

Using this approach, the corresponding 'nil residue' level was estimated to be in the order of $10^{-8}$ mg/kg, that is, 0.01 parts per trillion. Industry stakeholders should be aware that there is potential for a reduction in this withholding period, but that reduction would require the provision of additional residues data that address the limitations outlined in the previous paragraph.

### 5.4.8 Dimetridazole residues in poultry edible tissues

APVMA's Chemistry and Residues Program evaluated a series of residues trials conducted in turkeys (Ward 1964, Muggleton 1965, Anon 1965) and chickens (Muggleton 1963). The results from these trials indicate that when birds are treated at the maximum label rate of 500 mg dimetridazole/kg feed or 500 mg dimetridazole/L drinking water, residues in edible tissues decline to below the LOQ (less than 0.1 mg/kg) within two days of drug withdrawal. However, the data from these trials are of limited value, since a polarographic method that incorporated a benzene extraction step was used to assay the levels of dimetridazole residues in edible tissues from treated birds.

The validity of the residues results is dependent on the efficiency with which the residue components are extracted from tissues using benzene. Owing to the relatively high water solubility of the residue components, it is likely that the extraction efficiency is poor. Consequently, the residue data are unlikely to accurately reflect the levels of incurred residues in edible tissues.

In the absence of appropriate residues data for edible tissues, a meat withholding period may be extrapolated from the residue data for eggs. The distribution of residues into tissues, and subsequent residue depletion over time, is a function of both drug pharmacokinetics and metabolic processes.

During treatment, the drug circulates through the body, and partitions into tissues. During the elimination phase, the drug is metabolised and/or excreted, with tissue residues being remobilised then re-entering the blood.

Given that the albumen fraction of eggs is produced and excreted in the 24 hours before laying, the presence of any dimetridazole residues in plasma would be expected to result in the deposition of residues in albumen. Thus, when egg albumen residues are determined to be zero (after a 28-day withdrawal period), the levels of tissue residues are also expected to be zero. On the basis of this rationale, the meat withholding period should also be set at 28 days.

Industry stakeholders should be aware that there is potential for a reduction in this withholding period, but that any such reduction would require the provision of appropriate residues data that address the amended residue definition, and use a validated analytical method that has an LOQ of 0.001 mg/kg or lower.

### 5.4.9 Dietary risk assessment

Dietary intake assessments involve calculation of the dietary exposure levels (amount of treated food consumed × residue level), and comparison of these exposure levels with the ADI for chronic dietary exposure, and the ARfD for acute dietary exposure.

The dietary risk to human health from the use of dimetridazole in broiler breeders and breeder turkeys is considered to be nil, since the levels of dimetridazole residues in edible commodities from treated birds are expected to be zero, when the recommended meat and egg withholding periods are observed.

### 5.4.10 Residues-related aspects of trade

Risks to Australian trade from chemical residues arise when importing countries have not set tolerances for the chemical/drug residues in food commodities, or when

tolerances in the importing countries are lower than the corresponding Australian MRLs.

The use of dimetridazole in broiler breeders and turkey breeders, with meat and egg withholding periods of 28 days, is expected to result in a ‘nil residue’ situation. Therefore, this use pattern would not unduly prejudice Australia’s export trade in poultry commodities.

# 6. SUMMARY OF PUBLIC SUBMISSIONS

Eleven written submissions were received from State government departments, registrants and user groups. The concerns outlined in the submissions varied from repeated observations that there is no suitable alternative chemical treatment available to control blackhead in poultry (particularly turkeys) and that there is therefore considerable potential for an adverse economic impact on a range of business interests arising from this review, to the potential impact on human health, including worker safety particularly at the level of the end user.

Two submissions were received from State government departments stating that products containing dimetridazole are considered essential in both the poultry and pig industries. Alternative chemical treatments are available for the control of swine dysentery in pigs but no therapeutic agent is available for the prevention or treatment of blackhead in poultry. It was also noted that careful management controls have helped to reduce the occurrence of outbreaks of both these diseases but that outbreaks are a serious problem when they occur.

Four submissions from producer organisations argued that dimetridazole is an important chemical in the production of chicken and turkey meat in Australia. One submission included an estimate of the current economic impact from recent occasional outbreaks. The information provided supports the view of producer groups that there is likely to be a significant economic impact if the use of dimetridazole in chickens and turkeys is discontinued.

One submission indicated that there is an important off-label use of products containing dimetridazole to treat canker, caused by *Trichomonas spp.* in meat (squab) pigeons. The submission indicated that dimetridazole is primarily used in young birds kept for breeding purposes, and that the loss of dimetridazole for this use could have serious repercussions for the industry.

# 7. FINDINGS AND CONCLUSIONS OF THE REVIEW

## 7.1. Scheduling, safety directions and first aid instructions

Dimetridazole is currently listed in Schedule 4 of the SUSDP. The review concludes that the current entry should remain, in the event that registered veterinary products for companion animals and birds are sold on the Australian market. Furthermore, new safety directions are recommended (see Section 9 of this report) for dimetridazole while the current first aid instruction entry in the First Aid Instructions and Safety Directions Handbook is retained.

## 7.2 Use of dimetridazole in food-producing species

The review finds that there are insufficient toxicology data to support the existing ADI. As a result, the current ADI entry for dimetridazole will be deleted, because there is not an expectation of receiving new data to address these deficiencies. An ARfD for dimetridazole has not been established and is considered not applicable since the use of the chemical in food animals is not supported. Due to the lack of data, and safety concerns about genotoxicity, the registration of dimetridazole products for use in food-producing animals is not supported and will be withdrawn.

### 7.2.1 Use in pigs

It is unlikely to be economical or practical to routinely treat pigs with dimetridazole, as eventually all pigs are generally intended for human consumption, including breeding stock. Neither does any mechanism exist to distinguish between treated and untreated pigs. The review concludes that continued use of dimetridazole in pigs is not supported.

### 7.2.2 Use in commercial layers

Most products containing dimetridazole state on their label's withholding period instructions that the products should not be used in birds that are producing eggs, or may in the future produce eggs, for human consumption. However, a submission from egg producers indicated that at least one product containing dimetridazole may have been used in layers up to the point of lay.

Eggs containing residues of dimetridazole are unsuitable for human consumption. However, there may be a case for allowing a label claim for limited use of dimetridazole in commercial replacement chicken pullets and turkey poults if treatment ceases at least 28 days before the point of lay.

It is possible that commercial egg producers may wish to adopt this use pattern for replacement pullets that will produce eggs, or may in the future produce eggs, for human consumption.

Registrants would be required to make an application to the APVMA for this extended claim with relevant supporting data, which must include toxicology, OHS, residues and efficacy data packages for assessment. The application must also contain an industry plan for implementing the use pattern described in the previous paragraph. Stakeholders would need to demonstrate that their husbandry and management practices could accommodate the restrictions associated with such a use.

However, this use pattern will not be available to commercial producers until the use is approved on a product label. In the absence of such data and possible label approval, the use of dimetridazole in commercial poultry that are producing eggs, or may in the future produce eggs, for human consumption is not supported.

### 7.2.3 Use in breeding stock

There is currently no MRL entry in the MRL Standard for dimetridazole in eggs. Limited available data on residues in eggs from breeder poultry treated with dimetridazole suggest that the chemical should be non-detectable in meat and egg from these breeders 28 days after treatment. Analytical methods are available that can measure residue concentrations of dimetridazole and HMMNI as low as 0.001 mg/kg in meat and eggs.

The review concludes that in order to support the use of dimetridazole in breeders:

- an MRL of *0.001 mg/kg should be established for eggs;
- poultry producers are to observe a 28-day withholding period in order to supply eggs from breeder birds, and meat from culled breeder birds and spent hens, for human consumption with 'nil' residues; and
- the existing MRLs of dimetridazole for poultry meat and edible offal should be reduced from *0.005 mg/kg to *0.001 mg/kg.

### 7.2.4 Summary

Two strategies are proposed to support the ongoing limited use of dimetridazole in the poultry industry:

(i) supporting the use of the chemical in pigeons, caged birds and game birds not for human consumption; and

(ii) permitting continued, but limited use in breeder chickens and turkey breeders under still-to-be defined circumstances.

The APVMA recognises that dimetridazole is an important tool in the management of blackhead in poultry and that there is no registered alternative chemical available to treat outbreaks of this disease. Based on the residues assessment, there is a possibility that the APVMA may support the continued but limited use of dimetridazole in poultry breeding stock. As a review outcome, it is possible to support this approach because the human dietary exposure to meat and eggs from dimetridazole-treated breeder chickens and turkeys would be nil provided that label instructions are followed.

Assurance is required from industry, user groups and registrants to ensure that any recommended withholding periods for breeders, and restraints on using the chemical in non-breeding stock, will be observed. The APVMA intends to liaise with registrants, users and other key stakeholders to determine whether the use of dimetridazole in breeders could be supported. Should this outcome be supported, labels are to be varied with the label instructions outlined in Section 9.

## 7.3 Uses in non-food-producing species

The review finds that ongoing use in companion animals, including pigeons, caged birds and game birds, is supportable, provided that labels are varied with the label instructions outlined in Section 9 of this report. The toxicology assessment supports the registration of veterinary products for this use pattern and concludes that the approval of dimetridazole as an active constituent should continue.

The APVMA is aware that if controls exist or can be developed, the off-label use of dimetridazole products in breeding stock of squab pigeons would not be inconsistent with the finding of this review. Further, if dimetridazole is widely used in squab pigeons registrants should consider providing adequate information to the APVMA to enable product labels to be amended to reflect this claim for use.

In the absence of residue depletion data for dimetridazole in pigeons and game birds, the review concludes that the withholding periods recommended for breeder chickens and breeder turkeys must also apply to breeding stock of game birds and pigeons for squab production. Similarly, the restraints on using the chemical in non-breeding stock of chickens and turkeys intended for human consumption must also apply to non-breeding stock of pigeons and game birds intended for human consumption.

## 7.4 OHS risk mitigation

Veterinarians and other product users concerned about the lack of OHS information on product labels and the general absence of information about dimetridazole as a possible carcinogen also contacted the APVMA following the announcement of the review.

Although dimetridazole poses a genotoxic concern, the recommended safety directions (see Section 9) are considered sufficient to reduce the potential risks to workers from acute and repeated exposure to the chemical. Exposure to dimetridazole powder/dust could be further reduced if registrants were to package their products in water-soluble sachets. This type of packaging would negate the need for users to wear a respirator.

Both the APVMA and OCS (OHS) have had regard for the issues raised in the public submissions. Provided that the regulatory approach outlined in this review is adopted, and that the risk reduction mechanisms detailed on product labels are followed, it is concluded that the continued use of products containing dimetridazole would not be likely to have an effect that is harmful to human beings and would not be an undue hazard to the safety of people exposed to dimetridazole during its handling.

# 8. OVERSEAS REGULATORY STATUS

**United States**: In 1988, the US EPA announced the withdrawal of approvals for dimetridazole. The regulators in the United States considered that dimetridazole was demonstrated to have mutagenic effects on several bacterial strains and *Drosophila melanogaster* (fruit flies); that it caused a significant increase in the occurrence of mammary tumors in rats; that it is chemically similar to ipronidazole and metronidazole, chemicals that are considered to be carcinogenic in a mammalian species (mice); and that several metabolites of dimetridazole are suspected carcinogens. In addition, the US EPA considered that the available studies were inadequate (in terms of standards for such testing including the number of animals tested) and that there was an unacceptably high level of use of the chemical off-label in food-producing animals. Dimetridazole was not approved in the United States for use in pigs. The United States has continued to allow the use of dimetridazole under veterinary prescription, provided such use is confined to non-food-producing animals.

**European Union:** In July 1995, the EU withdrew the authorisation of dimetridazole as a veterinary medicine over concerns about its potential carcinogenicity. Although dimetridazole continued to be approved for use as a feed additive in the EU until 2001 the EU banned its use as a feed additive with effect from May 2002 on the basis that insufficient data had been submitted to meet the requirements of the re-evaluation. Note that dimetridazole continued to be used under licence as a feed additive in game birds in the United Kingdom until 2002, when supply was discontinued by the only registrant responsible for supply in the United Kingdom in response to the uncertain status of the chemical.

**Canada:** The Veterinary Drugs Directorate of Health Canada published amended regulations in August 2003 that withdrew the authorisation of all 5-nitroimidazole chemicals for use in animals that produce food for human consumption. The reasons given for this action related to the lack of sufficient toxicological information for bound residues of metabolic products. Note that the Veterinary Drugs Directorate continues to support the use of 5-nitroimidazoles in non-food-producing animals. The regulatory impact analysis statement released by Health Canada specifies that the use of 5-nitroimidazoles is permitted in some species, including ‘poultry birds’, provided adequate records are kept of treated animals and the animals are not sold for human consumption. Note that the use of 5-nitroimidazoles in Canada is not permitted in pigs as all swine are considered to be food-producing animals, whereas poultry birds can be food-producing animals or non-food-producing animals according to Health Canada.

**JECFA:** The Joint Expert Committee on Food Additives (JECFA), a joint body of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), reported that it was unable to set an ADI for dimetridazole when it considered the chemical in 1989. JECFA reported examining a study that showed an increase in the incidence of tumors in rats exposed to dimetridazole. By April 2002, JECFA reported that, in addition to inadequacies in the available data (in terms of standards for such testing), there was also insufficient information available to support the setting of an ADI or an MRL for dimetridazole due to a lack of data establishing the relative toxicological potency of bound residues and the absence of additional data establishing the relationship between a marker compound and the total residue.

In June 2002, JECFA concluded that dimetridazole is mutagenic *in vitro* but that available evidence is insufficient to determine if dimetridazole is mutagenic in animals. JECFA therefore requested that specific additional studies be undertaken to determine if dimetridazole is mutagenic in animals, and placed the chemical on the inactive list pending receipt and evaluation of additional human safety data.

**New Zealand:** The Agricultural Compounds and Veterinary Medicines Group of the New Zealand Food Safety Authority has included dimetridazole in a list of restricted substances and veterinary medicines in response to trade risks. Where a restricted veterinary medicine is used on food animals, conditions are imposed on the product registration unless tagging and tracking programs are instituted. A concern of New Zealand is the potential for off-label use of dimetridazole within the ostrich industry. The Animal Products Group of the New Zealand Food Safety Authority intended to address this concern by imposing additional restrictions on the overseas market access requirements for ostriches.

# 9. PROPOSED REGULATORY APPROACH

The APVMA proposes to find that it can vary the relevant particulars or the conditions of registration and approval for products containing dimetridazole and their labels listed in Appendix B of this report. These variations will enable some of the existing uses of dimetridazole to continue while complying with the requirements prescribed by the regulations for continued registration and approval. Further existing uses may be preserved subject to the APVMA being satisfied that the proposed withholding periods can be adhered to.

The APVMA proposes that:

- product labels be varied to specify that the use of dimetridazole is restricted to non-food-producing animals;
- product labels be varied to specify that the use of dimetridazole is permitted in broiler and turkey breeders, provided that the required assurances discussed in Section 6 are received;
- product labels be varied to include more detailed instructions for preparation and use;
- product labels be varied with new safety directions to ensure that users reduce their risk of exposure to dimetridazole; and
- the chemical dimetridazole be considered a possible genotoxic chemical.

The APVMA proposes to vary existing product labels with the following instructions.

1) Label claims that specifically restrict the use of dimetridazole to non-food-producing animals and breeders, such as:

   **'For use in breeding stock turkeys and other breeding stock of poultry, for the treatment and prevention of blackhead caused by *Histomonas meleagridis*', or similar.**

2) Under the DIRECTIONS FOR USE heading, insert as a restraint:

   **DO NOT USE in commercial laying hens, broilers or turkeys (meat birds);**

   Insert as an instruction:

   **For use in breeder chickens and breeder turkeys only;** and

   Insert as a warning:

   **Dimetridazole is a possible genotoxic chemical.**

3) Under the **Dosage and administration** subheading, dose instructions and other information in the directions for use are to be amended to clearly distinguish between regimes for treatment of infection versus instructions for prevention of infection.

   The recommended duration of treatment for each regime must be included in the instructions.

4) Under the heading **WITHHOLDING PERIODS,** repeat the restraint

**DO NOT USE in commercial laying hens, broilers or turkeys (meat birds).**

Retain the restraint:

**DO NOT USE in birds that are producing or may in the future produce eggs or egg products for human consumption.**

Insert the withholding periods:

**Meat: Meat from culled breeder birds and spent hens must not be made available for human consumption within 28 days of treatment.**

**Eggs: Excess eggs from breeder birds must not be made available for human consumption within 28 days of treatment.**

5) Under the heading **SAFETY DIRECTIONS,** from the First Aid Instructions and Safety Directions Handbook insert the standard statement codes 279, 280, 281, 290, 291b, 294, 298a, 300, 302, 360, 366, 361, 364. These statements translate to the personal protective equipment and safety directions:

**When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge). After each day's use wash contaminated clothing, gloves and respirator, and if rubber wash with detergent and warm water.**

For products containing dimetridazole and calcium lignosulfonate 20 g/kg or greater, hazard statement code 180:

**Repeated exposure may cause allergic disorders**

— is added to the above. The safety directions for these products are:

**Repeated exposure may cause allergic disorders. When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge). After each day's use wash contaminated clothing, gloves and respirator, and if rubber wash with detergent and warm water.**

# 10. IMPACT OF THE PROPOSED REGULATORY APPROACH AND AVAILABILITY OF ALTERNATIVE TREATMENTS

**Table 8. Summary of the anticipated impact of the APVMA regulatory approach, and registered alternative chemicals**

| Disease | Impact | Alternative |
|---|---|---|
| | **Registrants** | |
| Histomoniasis (blackhead) | Limitations on the use of dimetridazole in food-producing species may make products commercially non-viable as volume of sales decreases. | None is available. |
| Swine dysentery | Cancellation of a product that is used only in pigs is likely. | An alternative is available. |
| | **Producers of broiler breeders and breeder turkeys** | |
| Histomoniasis (blackhead) | Excess fertilised eggs and meat from spent hens may be marketed for human consumption.<br><br>There is need to observe lengthy meat and egg withholding periods.<br><br>Chickens and turkeys reared on litter are at increased risk disease. | There are no alternative registered chemical for treating blackhead. |
| | **Producers of commercial layer flocks** | |
| Histomoniasis (blackhead) | These producers are restrained from using dimetridazole.<br><br>Management of the pullets reared in cages, on litter and free range may require amendment so as to reduce the chance of the disease occurring.<br><br>Husbandry of sheds may need to be amended so as to control the caecal worm, which is the carrier of the causative disease agent. | There are no alternative registered chemical for treating blackhead. |
| | **Hobby poultry producers and others** | |
| Histomoniasis (blackhead) | Hobby producers are restrained from using dimetridazole if they are supplying meat and eggs for human consumption. | There are no alternative registered chemical for treating blackhead. |
| Trichomoniasis (canker) | The impacts are those described for niche industries. | Carnidazole and ronidazole are available. |

| Disease | Impact | Alternative |
|---|---|---|
| | **Niche Industries** | |
| Trichomoniasis (canker) | Loss of preferred option for group treating canker in pigeons.<br>Carnidazole is marketed for individual treatment but ronidazole is suitable for group treatment as it can be administered in drinking water.<br>Neither alternative can be used in birds intended for human consumption.<br>Ronidazole possesses mutagenic and carcinogenic properties.<br>The restrictions that are applied to turkeys and chickens are also applied to pigeons for squab production. | Two registered alternatives, carnidazole and ronidazole are available. |
| | **Pig Producers** | |
| Swine dysentery | There is little impact because there are several registered alternatives. | Tiamulin, tylosin, lincomycin and olaquindox are available. |
| | **Consumers** | |
| | Oral exposure to dimetridazole is reduced | Not applicable. |
| | **Workers and end-users** | |
| | Workers and users are required to wear personal protective equipment and observe safety directions so as to reduce dermal and inhalation exposure to dimetridazole. | Not applicable. |

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# APPENDIX A: Toxicology hazard profile of dimetridazole

### Absorption, distribution, metabolism and excretion in mammals

| | |
|---|---|
| Rate and extent of oral absorption | No data in laboratory animals; absorption is ≥75% in target animals (turkeys and pigs). |
| Distribution | No data in laboratory animals; in pigs, highest tissue concentrations (in descending order) occur in the liver, kidneys, lungs, spleen, fat and muscle. |
| Potential for accumulation | Not investigated. |
| Rate and extent of excretion | No data in laboratory animals; total excretion (urine, faeces and expired air) is rapid in turkeys (~90% within 72 h,), relatively slow in pigs (~75% in 7 days). |
| Metabolism | No data in laboratory animals; metabolism is extensive in turkeys, metabolism not adequately studied in pigs. |
| Toxicologically significant compounds (animals, plants and environment) | Parent compound; 1-methyl-5-nitroimidazol-2-yl methyl carboxylic acid (MNICA); 2-hydroxylmethyl-1-methyl-5-nitroimidazole (HMMNI); and other metabolites. |

### Acute toxicity

| | |
|---|---|
| Rat oral $LD_{50}$ (mg/kg bw) | 1600 |
| Worst oral $LD_{50}$ in other species | 1790 in mice |
| Rat dermal $LD_{50}$ (mg/kg bw) | No data |
| Worst dermal $LD_{50}$ in other species | No data |
| Rat inhalation $LC_{50}$ ($mg/m^3$) | No data |
| Worst inhalation $LC_{50}$ in other species | No data |
| Skin irritation | Appears to be non-irritant (limited data) |
| Eye irritation | Appears to be non-irritant (limited data) |
| Skin sensitisation | No data |

### Short-term and subchronic toxicity

| | |
|---|---|
| Target/critical effect | Rats: Toxic effects on testes, ovaries, stomach and heart at ≥200 mg/kg bw/d; central nervous system toxicity at 1,000 mg/kg bw/d. Dogs: central nervous system toxicity; skeletal muscle contraction; renal, liver, bone marrow and thyroid toxicity at ≥50 mg/kg bw/d po. Testicular toxicity at ≥90 mg/kg bw/d po. |
| Lowest relevant oral NOEL (mg/kg bw/d) | 40 in a 3-month study in dogs (but data not available for re-evaluation)*. |

| | |
|---|---|
| Lowest relevant dermal NOEL (mg/kg bw/d) | No data |
| Lowest relevant inhalation No Observed Effect Concentration ($mg/m^3$) | No data |

| | |
|---|---|
| **Genotoxicity** | Genotoxic in non-mammalian cells and possibly genotoxic in mammals. |

**Long-term toxicity and carcinogenicity**

| | |
|---|---|
| Target/critical effect | Liver toxicity at ~0.5 mg/kg bw/d po in rats. |
| Lowest relevant NOEL (mg/kg bw/d) | Could not be determined because of increased mortality and liver toxicity at ~0.5 mg/kg bw/d po (lower doses not tested). |
| Carcinogenicity | Benign mammary tumours in rats (dose-dependent; ≥15 mg/kg bw/day po; NOEL: 3.8 mg/kg bw/d, but data not available for re-evaluation); no data in a second species. |

**Reproductive toxicity**

| | |
|---|---|
| Reproduction target/critical effect | Decreased bw gains and food intake in F0 males and decreased lactation in F0 dams at ~200 mg/kg bw/d; increased mortality in F1b offspring at ~10 and 200 mg/kg bw/d (but data not available for re-evaluation)*. |
| Lowest relevant reproductive NOEL (mg/kg bw/d) | Could not be determined. |
| Developmental target/critical effect | Maternotoxicity: Reduced food intake and bw gains, and abortions at all doses (30-60 mg/kg bw/d).<br>Fetotoxicity: Death or total resorptions at 60 mg/kg bw/d; slight reduction in fetal weight (dose levels not stated). #,+ |
| Lowest relevant developmental NOEL (mg/kg bw/d) | Could not be determined. |

| | |
|---|---|
| **Delayed neurotoxicity** | No data |

| | |
|---|---|
| **Immunotoxicity** | No data |

| | |
|---|---|
| **Dermal absorption** | No data |

| **SUMMARY** | NOEL (mg/kg bw/d) | Study | Safety factor |
|---|---|---|---|
| **ADI** | Could not be determined | - | - |
| **ARfD (mg/kg/bw)** | Could not be determined | - | - |

| **Health value in drinking water** | Not necessary to set a health value. |
|---|---|

[#] Evaluation by JECFA (1990), study not evaluated by OCS.

[+] No data in a second species.

This profile for dimetridazole is developed from the OCS assessment of data submitted to the review and the OCS consideration of JECFA evaluations completed in 1990.
In addition, PACC has assessed acute toxicity studies in the rat, rabbit and guinea pig; reproductive toxicity in the pig, and metabolism studies in the rat and pig. However, the findings of these studies in Section 2 neither dispel the concern of dimetridazole's potential genotoxic carcinogenicity, nor give support for retention of its ADI.

# APPENDIX B: Dimetridazole products registered in Australia

| Product number | Registrant | Product name | Formulation | Registered uses |
|---|---|---|---|---|
| 35556 | Agribusiness Products Pty Ltd | Dimetridazole FG | Oral powder, premix | Swine dysentery in pigs |
| 38037 | Aventis Animal Nutrition Pty Ltd | Emtryl Premix for Feed Medication | Oral powder, premix | Swine dysentery in pigs<br>Blackhead in poultry and turkeys |
| 38038 | Aventis Animal Nutrition Pty Ltd | Emtryl Soluble Dimetridazole Soluble Powder 400g/kg | Soluble powder | Blackhead in game birds, poultry and turkeys<br>Trichomoniasis (canker) in pigeons<br>Swine dysentery in pigs |
| 50141 | Bronson and Jacobs Pty Ltd | Bronson and Jacobs Dimetridazole (D.M.Z.) Oral | Oral powder, premix | Swine dysentery in pigs<br>Blackhead in poultry and turkeys |
| 50743 | Ridley Agriproducts Pty Ltd | CCD Dimetridazole (Water Soluble Powder) | Oral powder, premix | Trichomoniasis (canker) in cage birds and pigeons<br>Blackhead in game birds, poultry and turkeys<br>Swine dysentery in pigs<br>General antifungal and antiprotozoan in poultry |
| 52812 | Ridley Agriproducts Pty Ltd | CCD DMZ 225 Premix (Dimetridazole) | Oral powder, premix | Swine dysentery in pigs<br>Blackhead in poultry and turkeys |