



Assessment and Prevention of Toxicity from Indospicine- contaminated horsemeat

**A report for the Rural Industries Research
and Development Corporation**

by Michael A. Pass

November 2000

RIRDC Publication No /2000...
RIRDC Project No USU-1A

© 2000 Rural Industries Research and Development Corporation.
All rights reserved.

ISBN 0 642 (...RIRDC to assign)
ISSN 1440-6845

Assessment and Prevention of Toxicity from Indospicine Contaminated Horsemeat
Publication no. /2000
Project no. USU-1A

The views expressed and the conclusions reached in this publication are those of the author and not necessarily those of persons consulted. RIRDC shall not be responsible in any way whatsoever to any person who relies in whole or in part on the contents of this report.

This publication is copyright. However, RIRDC encourages wide dissemination of its research, providing the Corporation is clearly acknowledged. For any other enquiries concerning reproduction, contact the Publications Manager on phone 02 6272 3186.

Researcher Contact Details

Dr Michael A. Pass
Faculty of Science, University of the Sunshine
Coast, Locked Bag No. 4, Maroochydore, DC, 4550

Phone: 07 5430 2840
Fax: 07 5430 2887
Email mpass@usc.edu.au

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au
Website: <http://www.rirdc.gov.au>

Published in November 2000
Printed on environmentally friendly paper by Canprint

Foreword

The Australian horsemeat industry is one of the country's newer export industries supplying markets in Asia and Europe. Retention and expansion of market share requires that the industry ensures its products are high quality in terms of their nutritive value, presentation and safety. An important safety issue relates to food products being free of chemical residues.

Australia has a reputation for producing high quality food free of chemical residues. The food quality is maintained through the meat inspection services and an extensive chemical-testing program to detect and mitigate contaminating residues. While the concentration of effort has traditionally focused on residues of veterinary drugs and agricultural chemicals, natural toxins are of emerging concern.

This report addresses the residue implications of one such natural toxin, the amino acid indospicine. This plant toxin has been reported to cause liver disease in dogs that have consumed indospicine-contaminated horsemeat and concern has been expressed for the safety of people who eat horsemeat. The research work reported here is an extension of a previous project supported by RIRDC. That project led to the development of assays for indospicine that are applicable to commercial situations. This project addresses the issue of the assessment of the risk to consumers of horsemeat that could be contaminated with indospicine.

The project investigated the mechanism of toxicity of indospicine with the view of providing information for determining the susceptibility of people to indospicine poisoning. This was aimed at providing a basis for assessing the risk of indospicine residues in horsemeat.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 450 research publications, forms part of our Horse R&D program, which aims to assist in developing the Australian horse industry and enhancing its export potential.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/pub/cat/contents.html

Peter Core
Managing Director
Rural Industries Research and Development Corporation

Acknowledgements

The author acknowledges the major contributions to this project made by Mrs Sandra Pollitt, Department of Physiology and Pharmacology, The University of Queensland and by Dr Mervyn P. Hegarty, Plantchem Pty, Ltd.

Contents

| | |
|---|------------|
| <i>Foreword</i> | <i>iii</i> |
| <i>Acknowledgements</i> | <i>iv</i> |
| <i>Contents</i> | <i>v</i> |
| <i>Executive Summary</i> | <i>vi</i> |
| | |
| 1. Introduction | 1 |
| 2. Objectives | 2 |
| 3. Methodological Approach and Results | 2 |
| 4. Discussion of Results | 4 |
| 5. Implications for Industry | 4 |
| 6. Recommendations | 5 |
| 7. Intellectual Property | 5 |
| 8. Communication Strategy | 5 |
| 9. References | 5 |

Executive Summary

Australian primary producers provide high quality, nutritious food to Australia and many other regions of the world. An important challenge to these industries is to minimise the occurrence of potentially toxic residues in food products to levels below those that would be injurious to health.

Australia has a long history of producing high quality beef and lamb for domestic and international consumption. In recent years, other meat-producing industries have developed and one of these is the export horsemeat industry. This has grown to be worth in excess of \$26 million annually. Being a relatively small industry, imposition of trade embargos by meat importing countries would have a significant effect on the viability of the industry. The occurrence of excessive chemical residues in food has been a common cause of trade embargos that have affected Australia's meat industries. Therefore, the significance of chemical residues to human health must be understood so that assessments can be made of acceptable residue limits in horsemeat if this industry is to be properly protected from unfair trade embargos.

For many years, Australia's food producing industries have concentrated their efforts on minimising residues of veterinary drugs and agricultural chemicals. In more recent years there has been a growing awareness that natural products produced by plants also might be harmful if they contaminate foodstuffs. It has been considered unusual for natural toxins to accumulate to significant levels in apparently healthy animals and little emphasis has been placed on the significance of natural toxins as residue risks. There is evidence however, that some natural toxins may accumulate in animal tissues and pose a risk to consumers and therefore to the industry from which the product is derived.

One of these toxic natural products is the amino acid indospicine that accumulates in the meat of horses grazing *Indigofera* plants in Australia. The major plant species involved is *Indigofera linnaei*, commonly called Birdsville Indigo. It grows extensively in Western Queensland and the Northern Territory. Consumption of indospicine-contaminated meat by dogs has resulted in liver disease that in some cases has been fatal. The potential toxicity for humans is unknown and therefore a risk analysis for people cannot be done. The horsemeat industry has made significant efforts to reduce the risks of indospicine-contaminated meat from entering the marketplace by sourcing animals from regions free of Birdsville Indigo. Nevertheless, the potential for contaminated meat reaching the marketplace still exists and there is a need for estimating the risk posed by indospicine residues in horsemeat.

The assessment of maximum residue limits of chemicals in food is complex and requires knowledge of the susceptibility of individuals to poisoning, the intake patterns of the food in question and the biology of acute and chronic toxicity caused by the chemical in question. This report describes a study on the mechanism of indospicine-induced liver disease that was aimed at providing a basis for risk assessment in people.

Research by several groups over the past half century has identified three possible explanations for the toxic action of indospicine. They include the toxin inhibiting protein synthesis, inhibiting the enzyme arginase or inhibiting the enzyme nitric oxide synthase (NOS). The current study concentrated on the role of indospicine as a NOS inhibitor as this mechanism has been shown to be the mechanism of liver disease caused by several other toxic chemicals and indospicine has been shown to inhibit NOS in other tissues.

An interesting feature of indospicine toxicity is that there are distinct differences in susceptibility of species to poisoning. For instance, dogs appear to be the most susceptible species, horses are resistant to poisoning and cattle, rats and rabbits appear to lie between these species. It was predicted that, if the reason for these differences was known, then the susceptibility of people to poisoning could be estimated and this would provide a basis for assessing the risk of poisoning to consumers of horsemeat.

Two hypotheses were tested in these experiments. One was that differences in susceptibility to poisoning was related to the amount of NOS in the livers of the different species and the second is that indospicine inhibits NOS in the liver similar to the inhibition seen in other tissues. A method was developed for detecting the activity of NOS in liver tissue and then the activities in liver collected from horses, dogs, rats and rabbits were compared. No differences were found in NOS activity between the species tested indicating that differences in NOS activity is not the basis of the differences in species susceptibilities to indospicine poisoning.

Studies on the effect of indospicine on hepatic NOS activity demonstrated that indospicine had only a small effect on hepatic NOS. There was an indication that indospicine may increase NOS activity and therefore nitric oxide (NO) synthesis under some conditions. Depending on the particular situation, NO can be cytotoxic or cytoprotective and therefore, the possibility exists that indospicine-induced liver disease may be a result of increased NO synthesis rather than decreased synthesis as previously suggested.

The results of this project have not given a clear indication of the mechanism of indospicine poisoning, although they indicate that differences in hepatic NOS are not the basis for the species differences in susceptibility to the toxin. Clearly more research is required before a risk assessment for people can be made and the significance to human health of indospicine residues in horsemeat determined.

1.Introduction

Australia has a reputation for producing safe foods free of chemical residues. The quality of our primary products is assured through the excellent methods for drug and chemical residue surveillance that have been established in this country.

By necessity, the residue concerns have concentrated on the detection of veterinary drugs and agricultural chemicals. More recently, concerns have arisen about contamination of food with natural toxins and the implications for human health and the meat export trade. One of the toxins of concern is indospicine and this report describes research aimed at improving our understanding of the biology of this toxin and its significance for human health.

Indospicine is a naturally occurring toxic amino acid that is structurally related to arginine. When horses consume plants containing indospicine, the toxin accumulates in their tissues including the muscle meat. When the meat is consumed by dogs it causes fatal liver necrosis (Hegarty *et al* 1988) and a significant potential exists for toxicity in other species including people. In Australia, the plant implicated in this toxicity is *Indigofera linnaei*, commonly known as Birdsville Indigo that grows in grazing pastures in Western Queensland and the Northern Territory.

Indospicine has been shown to cause liver disease in a number of species including dogs, cattle and rats (Hegarty, 1978; Hegarty *et al* 1988). An important feature of this disease is that indospicine accumulates in the meat of horses that eat Birdsville Indigo and is transmitted to other animals that eat the meat (Hegarty *et al* 1988). As mentioned above, indospicine consumed in horsemeat causes fatal liver injury in dogs (Hegarty *et al* 1988). Since liver injury associated with indospicine is not confined to dogs, a potential also exists for such injury in people particularly if the intake is sufficiently high and prolonged.

The consequences of the existence of indospicine in horsemeat are two-fold:

- 1) Consumption of contaminated horsemeat could lead to toxicity in those people eating the meat or in animals to which the meat is fed.
- 2) The potential or perceived potential for indospicine toxicity in consumers of horsemeat is a real threat to horsemeat exports from Australia. The toxicity and its transmission through meat are well-recorded (Hegarty *et al* 1988) and is known to scientists internationally who are concerned with natural toxins and toxic residues in food. A meeting of concerned scientists coordinated by the Australian Bureau of Rural Resources, concluded that indospicine is one of the most important naturally occurring toxic contaminants in meat that could adversely affect the export of meat from Australia (Nicholls 1993).

The regulatory procedures associated with residue prevention include assessment of the risk potential of the chemical, the availability of analytical techniques for monitoring the presence of the chemical, sampling procedures to maximize the chance of economically detecting contamination and trace back procedures to detect the source of contaminated animals. RIRDC has supported the development of analytical methods for monitoring indospicine in horsemeat including an HPLC method and an ELISA (Pass 2000; Pollitt *et al* 2000). The potential for human toxicity is still undetermined as direct testing cannot be performed and extrapolation of data from other species is unreliable because of the species differences in susceptibility to the toxic effects of indospicine. For instance, dogs appear most susceptible to poisoning, but horses appear resistant despite high concentrations of the amino acid

accumulating in the liver of horses grazing Birdsville Indigo. Other species such as cattle, rats and rabbits show intermediate susceptibility. As well as these species differences, there are also individual differences within a species. For example, some dogs are much more susceptible than others to indospicine toxicity (Young 1992).

Although this species variability makes it difficult to assess potential human susceptibility by direct extrapolation, it does provide a potential avenue for such assessment. It was proposed that if the mechanism responsible for the species differences in susceptibility is known, then it would be possible to determine to what extent this operated in people. This would then allow a determination of the potential susceptibility for human intoxication and form a basis for risk assessment. This was the basis of this project.

Indospicine causes liver damage characterised by necrosis of liver cells and ultimately liver failure (Hegarty 1988; Kelly *et al* 1992; Young 1992). Three suggestions have been advanced to explain the mechanism of toxicity. They are:

- 1) Indospicine has been shown to be an inhibitor of protein synthesis and through this, an inhibitor of DNA synthesis (Christie *et al* 1969; Christie *et al* 1971; Madsen *et al* 1970). It was suggested that this action could be responsible for liver cell injury. However, it is difficult to explain the species differences in susceptibility by this mechanism since all animals must synthesise protein and DNA to remain viable.

- 2) Indospicine is structurally related to arginine and competitively inhibits a range of biochemical reactions involving arginine including the metabolism of arginine by the enzyme arginase (Madsen and Hegarty 1970). It was suggested that susceptibility to poisoning could be due to a disturbance to ammonia metabolism as a result of inhibition of arginase (Madsen and Hegarty 1970)

- 3) Nitric oxide (NO) is an important regulator of many cell functions. It is synthesised from arginine by nitric oxide synthase (NOS). Arginine analogues including indospicine inhibit NOS and thus reduce NO synthesis (Pass *et al* 1996). NO has been shown to protect the liver from superoxide-induced injury and NOS inhibitors have been shown to enhance hepatotoxicity caused by a number of chemicals (Billiar *et al* 1990; Harbrecht *et al* 1992a; Harbrecht *et al* 1992b; Kuo and Slivka 1994; Kuo and Abe 1995; Nanji *et al* 1995; Muriel 1998). Since indospicine is a known inhibitor of NOS in some tissues, it was hypothesised that indospicine-induced hepatotoxicity may be a result of NOS inhibition with consequent increased superoxide-mediated injury. Thus, the species differences in susceptibility may be due to the relative importance of NOS in protection against superoxide-mediated injury in the different species.

Since the NO hypothesis offered an explanation of how species differences in susceptibility to indospicine-induced liver injury might occur, it was decided to investigate this mechanism further. Two investigations were carried out. One was to compare hepatic NOS activity in different species and the second was to investigate the effect of indospicine on hepatic NOS.

2. Objectives

The objective of the project was to test the following hypotheses:

- 1) That species with different susceptibilities to indospicine toxicity differed in their hepatic NOS activities.
- 2) That indospicine is an inhibitor of hepatic NOS.

3. Methodological Approach and Results

Hepatic NOS exists in two forms, a constitutive form (cNOS) and an inducible form (iNOS). cNOS occurs in low concentrations and it is the iNOS that appears to be responsible for protection against superoxide-induced hepatotoxicity. To ensure that the method chosen actually detected and measured iNOS, an experiment was performed in which rats were treated with endotoxin to induce hepatic iNOS and the activity of the enzyme in treated rats livers was compared with that in untreated control animals. Hepatic iNOS activity was measured by the amount of NO synthesised by the enzyme as estimated by the oxidation of oxyhaemoglobin to methaemoglobin (Feelish and Noack 1987; Noack *et al* 1992). All estimations were performed on liver homogenates prepared from livers perfused with enzyme inhibitors to prevent deterioration of iNOS prior to estimation of its activity.

The results of this experiment indicated that iNOS activity was not present in control rat livers and that endotoxin induced hepatic iNOS activity to 4.3 ± 2.3 nmol NO synthesised/min/g liver (mean \pm SD, n=7). It was concluded that the technique was sufficiently sensitive for the estimation of iNOS activity in liver tissue and for estimating the effect of indospicine on iNOS activity.

Experiments were conducted to estimate iNOS activity in the livers of horses, dogs, rats and rabbits. Liver samples were collected from animals immediately after slaughter and perfused with a buffered electrolyte solution containing several enzyme inhibitors to prevent the degradation of iNOS. Liver homogenates were prepared and the iNOS activity estimated by the method described above. iNOS activity was not detected in liver homogenates from any of the animals. This was in contrast to the activity detected in livers from endotoxin treated rats.

The effect of indospicine on hepatic iNOS activity was estimated using liver homogenates prepared from rats treated with endotoxin. In contrast to studies that demonstrated that indospicine-mediated inhibition of cNOS and iNOS in other tissues (Pass *et al* 1996), indospicine caused little inhibition of iNOS activity in liver homogenates and only at relatively high concentrations of the amino acid. This was in contrast to the arginine analogues L-canavanine and L-nitroarginine methyl ester (L-NAME), that caused approximately, 60 and 85% inhibition respectively (Table 1).

Table 1 – Effect of arginine analogues on iNOS activity (mean of n=3).

| Treatment | iNOS activity (% of control) |
|--------------------|------------------------------|
| Control | 100 |
| mM L-canavanine | 38.6 |
| 1 mM L-NAME | 15.8 |
| 1 mM L-indospicine | 99.0 |
| 10mM L-indospicine | 88.2 |

Interestingly, in a single experiment, it appeared that indospicine may have increased *i*NOS activity when the toxin was pre-incubated with the homogenate for 45 min (Fig 1).

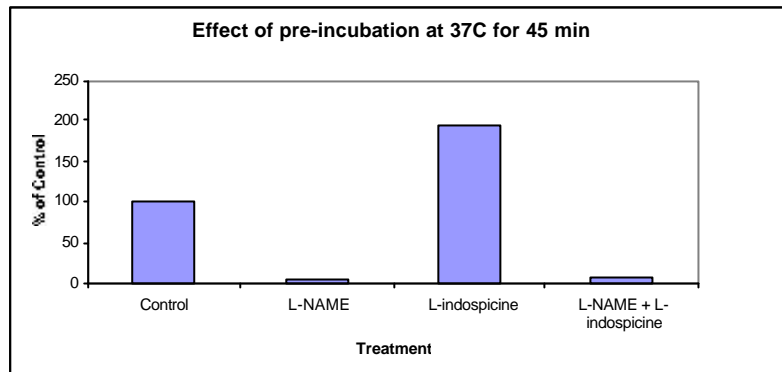


Fig 1 – Effect on *i*NOS activity in a liver homogenate after pre-incubation with arginine analogues.

4. Discussion of Results

Unfortunately, the results of this study do not provide an explanation for the species differences in the susceptibility to indospicine toxicity that is required for making an assessment of the risk to people of this intoxication. Indospicine was a poor inhibitor of hepatic *i*NOS compared to other arginine analogues and *i*NOS was not detected in the livers of the animals studied. This was in contrast to the enzyme activity in rats treated with endotoxin, an inducer of hepatic *i*NOS. Ten dogs and 10 horses were studied and the results indicate that induction of hepatic *i*NOS is not common in animals kept in ordinary environments. Although dogs appear to be the species most susceptible to indospicine toxicity, there is great variability in susceptibility among individual animals. It is interesting to speculate that susceptibility may only occur in animals in which hepatic *i*NOS has been induced. Indeed, in experimental animals, NOS inhibitor-induced hepatotoxicity required *i*NOS induction prior to the exposure to the hepatotoxic agent. Since *i*NOS was not detected in the dog livers studied, it was not possible to investigate the effect of indospicine on dog hepatic *i*NOS.

The increase in *i*NOS activity and NO synthesis in the liver homogenate pre-incubated with indospicine was interesting, and suggests an avenue for further investigation. Why this increase occurred is unclear but it could have relevance for indospicine toxicity. NO is known to be both cytotoxic and cytoprotective depending of the particular environment in which it is operating. For instance, NO has the capacity to react with superoxide to “neutralise” superoxide and prevent cytotoxicity induced by superoxide. Alternatively, NO can also react with superoxide to produce peroxynitrite that is cytotoxic. If the latter occurred as a result of increased NO synthesis induced by indospicine, then cytotoxicity could result.

Clearly there is much more to be learned about the mechanism of indospicine poisoning and the reasons why some animals are susceptible to poisoning and others are resistant. The current results indicate that differences in *i*NOS activity in the liver is not the reason for susceptibility differences. Other possible modes of action of the toxin such as its potential for increasing NO synthesis are areas worth pursuing in future studies.

5. Implication for Industry

Chemical contamination of meat has serious health implications as well as implications for public confidence in the food producing industries and for trade. To ensure food is free of chemical contamination, reliable methods for detection and quantitation of chemicals in food are required and an understanding of the biology of the compound is required to establish the risk of intoxication. Of the toxic natural products that contaminate meat, indospicine has been shown to cause toxicity when horsemeat contaminated by natural acquisition of the amino acid is consumed by another species. Commercially applicable analytical methods for detecting indospicine in meat have been developed (Pass 2000; Pollitt *et al* 2000). However, an understanding of the biology of indospicine that will allow a risk assessment for human intoxication is still incomplete and further research is required.

Research of this type is, by its very nature, an insurance against future problems for the industry and a cost benefit analysis is difficult to make at this stage. The results have eliminated one explanation for indospicine toxicity and have thus brought us closer to understanding the biology of this disease. The investment was small and it is likely that similar modest investments in the future will bring this area of research to a suitable conclusion.

6. Recommendations

Minimizing the effect of residue incidents on an industry depends on having adequate procedures for detecting residues and on the capacity to provide a risk assessment for toxicity of the chemical in people. Previous funding by the RIRDC has supported the development of analytical procedures for the detection of indospicine residues and this has been reported previously (Pass, 2000). However, the problem of risk assessment remains. Our level of understanding of the biology of indospicine including the factors responsible for toxicity, is still inadequate for a proper risk assessment to be made and further work is required. It is for the industry to decide whether this research should be pursued. Hopefully, this decision will be based on the perceived risk to human health posed by indospicine and the risk to the export market should indospicine residues be detected in horsemeat.

7. Intellectual Property

The information produced has no direct commercial value and its value resides in the scientific merit of the information.

8. Communication Strategy

The work will be incorporated in a PhD thesis being prepared by Mrs Sandra Pollitt (Pollitt, S. (2001) Residue Implications of Indospicine. PhD Thesis, The University of Queensland). The thesis encompasses this work and the research that developed the analytical procedures for detecting indospicine in horsemeat. Results of this research will be presented at an appropriate conference.

9. References

- Billiar, T. R., Curran, R. D., Harbrecht, B. G., Stuehr, D. J., Demetris, A. J., and Simmons, R. L. (1990). Modulation of nitrogen oxide synthesis in vivo: N-monomethyl-L-arginine inhibits endotoxin-induced nitrite/nitrate biosynthesis while promoting hepatic damage. *Journal of Leukocyte Biology* 48, 565-569.
- Christie, G. S., De Munk, F. G., Madsen, N. P., and Hegarty, M. P. (1971). The effects of an arginine antagonist on stimulated human lymphocytes in culture. *Pathology* 3, 139-144.
- Christie, G. S., Madsen, N. P., and Hegarty, M. P. (1969). Acute biochemical changes in rat liver induced by the naturally occurring amino acid indospicine. *Biochem. Pharmacol.* 18, 693-700.
- Feelisch, M., and Noack, E. A. (1987). Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *Eur. J. Pharmacol.* 139, 19-30.
- Harbrecht, B. G., Billiar, T. R., Stadler, J., Demetris, A. J., Ochoa, J., Curran, R. D., and Simmons, R. L. (1992a). Inhibition of nitric oxide synthesis during endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. *Journal of Leukocyte Biology* 52, 390-394.
- Harbrecht, B. G., Billiar, T. R., Stadler, J., Demetris, A. J., Ochoa, J. B., Curran, R. D., and Simmons, R. L. (1992b). Nitric oxide synthesis serves to reduce hepatic damage during acute murine endotoxemia. *Critical Care Medicine* 20, 1568-1574.
- Hegarty, M. P. (1978). Toxic amino acids of plant origin. In, *Effects of Poisonous Plants on Livestock*. (eds. Keeler, RF, Van Kampen, KR and James, LF) Academic Press, New York. pp. 575-585.
- Hegarty, M. P., Kelly, W. R., McEwan, D., Williams, O. J., and Cameron, R. (1988). Hepatotoxicity to dogs of horse meat contaminated with indospicine. *Australian Veterinary Journal* 65, 337-340.
- Kelly, W. R., Young, M. P., Hegarty, M. P., and Simpson, G. D. (1992). The hepatotoxicity of indospicine in dogs. In, *Poisonous Plants: Proceedings of the Third International Symposium*. (eds. James, LF, Keeler, RF, Bailey, EM, Cheeke, PR and Hegarty, MP) Iowa State University Press, Ames, Iowa. pp. 126-130.
- Kuo, P. C., and Abe, K. Y. (1995). Interleukin 1-induced production of nitric oxide inhibits benzenetriol-mediated oxidative injury in rat hepatocytes. *Gastroenterology* 109, 206-216.
- Kuo, P. C., and Slivka, A. (1994). Nitric oxide decreases oxidant-mediated hepatocyte injury. *Journal of Surgical Research* 56, 594-600.
- Madsen, N. P., Christie, G. S., and Hegarty, M. P. (1970). Effect of indospicine on incorporation of L-arginine-14C into protein and transfer ribonucleic acid by cell-free systems from rat liver. *Biochem. Pharmacol.* 19, 853-7.

- Madsen, N. P., and Hegarty, M. P. (1970). Inhibition of rat liver homogenate arginase activity in vitro by the hepatotoxic amino acid indospicine. *Biochem. Pharmacol.* 19, 2391-3.
- Muriel, P. (1998). Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation, and liver damage induced by carbon tetrachloride. *Biochemical. Pharmacol.* 56, 773-779.
- Nanji, A. A., Greenberg, S. S., Tahan, S. R., Fogt, F., Loscalzo, J., Hossein Sadrzadeh, S. M., Xie, J., and Stamler, J. S. (1995). Nitric oxide production in experimental alcoholic liver disease in the rat: Role in protection from injury. *Gastroenterology* 109, 899-907.
- Nicholls, T. (1993). Report of the Select Meeting of Plant Toxins and Miscellaneous Chemicals; Held for the Sub Committee on Residues in Animal Products. Australian Bureau of Rural Resources, Canberra.
- Noack, E., Kubitzek, D., and Kojda, G. (1992). Spectrophotometric determination of nitric oxide using hemoglobin. *Neuroprotocols* 1, 133-139.
- Pass, MA (2000) Contaminated Horsemeat – Assessment and Prevention of Toxicity from Indospicine. RIRDC Publication No. R00/011.
- Pass, M. A., Arab, H., Pollitt, S., and Hegarty, M. P. (1996). Effects of the naturally occurring arginine analogues indospicine and canavanine on nitric oxide mediated functions in aortic endothelium and peritoneal macrophages. *Natural Toxins* 4, 135-40.
- Pollitt, S, Hegarty, MP and Pass, MA (2000) Analysis of the amino acid indospicine in biological samples by high performance liquid chromatography. *Natural Toxins*, In press.
- Young, M. P. (1992). Investigation of the toxicity of horsemeat due to contamination by indospicine. PhD thesis, The University of Queensland.