Determination of a No Observed Effect Level for corynetoxins

Summary
Corynetoxins cause annual ryegrass toxicity (ARGT) and have the potential to contaminate grains.

Following extensive trial studies, the 12 month No Observed Effect Levels (NOELs) of dietary tunicamycins were determined for clinical and pathological effects in rats and sheep of both genders. The tunicamycins were used as a model for the more difficult to obtain corynetoxins.

The toxicological equivalence of tunicamycins and corynetoxins under these chronic exposure conditions was confirmed in a separate study in rats.

The reversibility of effects following six months of dietary exposure to tunicamycins was examined in male rats over a six month recovery period.

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Conclusions
In a carefully designed and monitored project, purified and fully characterised commercially-provided tunicamycins were orally administered to sheep and rats of both genders via their diet for a period of 12 months. Clinical, behavioural, biochemical, pathological and histopathological data were used to determine the Lowest Observed Effect Levels (LOELs) and the No Observed Effect Levels (NOELs) for those criteria. The project confirmed a cumulative nature to the exposure of rats and sheep to tunicamycins and corynetoxins in their diets.

Assessment of the data from both genders of both species provided an overall NOEL of approximately 1.5 or 12g tunicamycins/kg bodyweight/day for 12 months for the effects on the liver rough microsomal N-acetylglucosamine-1-P-transferase or the liver pathology, respectively. Similarly, the overall LOEL was estimated at 4.5 or 37g tunicamycins/kg bodyweight/day for 12 months for the effects on the liver rough microsomal N-acetylglucosamine-1-P-transferase or the liver pathology, respectively.

The use of tunicamycins to model exposure to corynetoxins under these long-term dietary exposure conditions was validated. Therefore, the NOEL data derived for tunicamycins can be extrapolated to corynetoxins with confidence.

Only the testicular lesions were irreversible under the conditions used in this study.

Recommendations
This data should be made available to Food Standards Australia New Zealand (FSANZ) for assessment and to enable the determination of tolerable levels of dietary exposure to corynetoxins for humans.

The data should then be used to help develop measures that will protect the consumer from low-level, long-term exposure to the corynetoxins in grains and grain products. These measures will also help protect against non-tariff trade barriers that may be implemented by international customers.

Outcomes
A NOEL for 12 months dietary exposure to corynetoxins will be established based upon observations of various clinical and pathological effects in rats and sheep of both genders.

This NOEL will provide basic toxicology information to food safety authorities and enable the determination of a lowest tolerable level of corynetoxins in food for human consumption.
Economic outcome

The grains industry will be able to defend against non-tariff trade barriers based upon the potential for contamination by corynetoxins.

Environmental outcome

Grains can be screened for the appropriate levels of corynetoxins. Growers with higher than acceptable levels will be encouraged to engage in corynetoxin reduction practices.

Social outcome

The safety of food for human consumption, and feed for animal consumption, will be monitored with respect to the presence of corynetoxins. Levels below the appropriate NOEL will be safe for human or animal consumers of grains and grain products.

Achievements/Benefits

Corynetoxins are a group of naturally occurring tunicamyluracil glycolipids that cause the neurologically-manifested intoxication called annual ryegrass toxicity (ARGT).

Corynetoxins are produced by the bacterium Rathayibacter toxicus that colonises the seed heads of annual ryegrass (Lolium rigidum). The colonisation is enabled by a nematode (Anguina funesta) that itself colonises the seed heads as part of its reproductive cycle. If the nematode carries the bacterium along with it to the seed head and the subsequent nematode gall within the seed head, then the bacterium can outcompete the nematode producing a golden-coloured ‘bacterial gall’. The bacterial colony produces the corynetoxins as the plant begins to senesce. The antibiotic properties of the corynetoxins suggest that the biosynthesis may be part of a survival strategy for R. toxicus, eliminating other competitors for the diminishing plant resources as the plant dries.

One of the primary biochemical effects of corynetoxins (and tunicamycins) is the inhibition of the enzyme N-acetylglucosamine-1-phosphate transferase (GPT) that is involved in the assembly of N-linked glycans before their transfer to nascent proteins in the endoplasmic reticulum.

Annual ryegrass is used as a pasture improvement plant providing valuable feed for sheep. However, it can contaminate crops destined for human food and animal feed thereby providing the potential for contamination of those crops by corynetoxins. It is the potential for contamination of grains that prompted the GRDC to seek the opinion of the Australia New Zealand Food Authority (ANZFA), now the Food Standards Australia New Zealand (FSANZ) on the likely levels of corynetoxins that could be tolerated in grains.

After a complete review and assessment of the literature, ANZFA concluded (September 1997) that there were insufficient data to define the levels of acceptable contamination. The report stated that “In conclusion, assessment of the potential risk associated with exposure to corynetoxins will require further research, with respect to both the toxicological properties of corynetoxins and also the distribution of residues of corynetoxins in foods. This research should be given a high priority”.

Consequently, the CSIRO Plant-Associated Toxins Research Group conducted several toxicology studies to provide the toxicological data required by food safety authorities to estimate tolerable exposure levels of corynetoxins.

Following extensive trial studies and based upon decades of prior experience with ARGT, the tunicamycins and the corynetoxins, the project team determined the NOELs of tunicamycins on various clinical and pathological parameters in sheep and rats of both genders. The study was designed with due cognisance of the Organisation for Economic Co-operation and Development (OECD) guideline for conducting such toxicology studies.

The tunicamycins were used to model the effects of corynetoxins based upon the proven toxicological bioequivalence at more acute exposures. This bioequivalence was confirmed during a 12 month study in which male and female rats were exposed to tunicamycins or corynetoxins in their diet. The confirmation thereby validated the tunicamycins model of exposure to corynetoxins under the chronic, low-level exposure conditions.

The potential for reversibility of effects was investigated in a study in which male rats were exposed to dietary tunicamycins
for six months and then given access to toxin-free feed for another six months. Most clinical, biochemical and pathology effects were reversed in the recovery period. Only the degenerate testes did not recover, nor showed any signs of recovery, within the six month recovery period.

Other research

This project poses other questions (and hence research projects) that may assume priority in future assessments of the food safety issues associated with contamination of grains with corynetoxins i.e.

1. Differences in species susceptibilities. How can the data be extrapolated to humans?
2. What level of GPT inactivation constitutes an adverse effect?
3. Based upon testicular effects and related work with pregnant rats, a full developmental reproductive study may be required.

Once an acceptable level of corynetoxins has been established for grains, research should be targeted at adapting the CSIRO/GRDC-funded corynetoxin ELISA for rapid ‘Yes/No’ screening of all grains entering the food supply.

Intellectual property summary

Data are protected by the CSIRO records management system. No commercialisation is applicable.