Reducing the threat Tutu toxic tutu honey poses to the New Zealand beekeeping industry and consumers

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Reducing the threat Tutu toxic honey poses to the New Zealand beekeeping industry and consumers

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This report presents the results of studies on the levels of toxins in honey samples and the toxicity of tutin to mammals. Results of the analyses for 20 'at-risk' honey samples are tabulated. No tutin was detected in the honey samples. However, hyenanchin, a metabolite of tutin, was found in 13 of the 20 samples tested. The highest level found was 2.6 mg hyenanchin/kg of honey. Mice were fed pure tutin, the priary toxic compound in the tutu plant. The toxicity for tutin was determined and reported as the lethal dose required to kill 50% of the trial animals (LD_{50}) The minimum dose at which there is no-observable adverse effect level (NOAEL) is also included. The NOAEL for tutin in fed mice was 0.25 mg/kg. From this, by application of a safety factor of 100, an ARfD of 0.15 mg was calculated for a 60 kg man

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INTRODUCTION

Toxic honey produced by bees collecting honey from tutu plants has been a well recognised problem for the beekeeping industry in New Zealand for over 60 years. Tutu contains the toxic compound tutin, which can be metabolised into hyenanchin. Todate, hyenanxhin has been found in honey-dew and honey. (Appendix 1).

The work undertaken for this report aimed to provide the beekeeping industry with information on the current levels of tutin and hyenanchin in examples of potentially toxic honey so that the industry may assess the current management strategies for dealing with the risk These honey samples came from the Coromandel and Bay of Plenty areas that have traditionally been regions where toxic honey has been produced.

ANALYSIS OF HONEY SAMPLES

Initially, when honey was being tested in New Zealand about 60 years ago, the testing of suspected toxic honey was carried out using small animals – rats, mice and guinea pigs (Clinch & Turner 1968). While the testing did provide some information about the toxicity of the honey, the testing was neither specific nor was it suitable for routine rapid screening of honey samples. An additional confounding factor was that humans were noted to be more sensitive to tutin than rats or guinea-pigs (Palmer-Jones & White). In 1965, thin layer chromatography (TLC) started to replace animals as the test. The accuracy of toxic honey testing improved in 1986 when liquid chromatography (LC) methodology utilising UV detection was developed (Love et al. 1986). However, the specificity of the testing improved to a robust level only when mass spectroscopy (MS) detection became available (reference?).

ESTABLISHMENT OF TUTIN TOXICITY LEVELS

The lethal doses measure of toxicity is remains fundamental in to the understanding the scope of the toxic honey threat. Dr Rex Munday of AgResearch was contracted to establish the lethal dose (LD_{50}) in mice and the minimum amount of tutin that may be ingested not suffering any effects (NOAEL). See Appendix 1 for his report.

MATERIAL AND METHODS

HONEY SAMPLES AND TESTING

Prior to the start of this SFF contract, HortResearch had developed an improved method for the analysis of tutin and hyenanchin in honey, separating the components by liquid chromatography and detecting the analytes with mass spectroscopy (LCMS). The method was based on established published methods but applied more modern clean-up techniques. The advantage of using LCMS for this analysis is the ability to improve accuracy and maximise sensitivity by screening out any chemical noise from other compounds in the honey.

The National Beekeepers Association of New Zealand arranged for the delivery to HortResearch of honey samples for analysis from selected members of the Association who harvested honey from the potential 'at-risk' areas. Samples came from eastern Bay of Plenty, both sides of the Coromandel Peninsula and Waikato. Twenty of the 28 samples received from five different suppliers were selected and analysed. They were thoroughly mixed and diluted with water and purified before LCMS analysis liquid chromatography separation and measurement by mass spectroscopy. Each supplier was provided with their results.

To validate each set of analyses, an additional honey sample that had been spiked with the analytes (tutin and hyenanchin) is included in the batch of samples. The honey was spiked at a rate of 4.8 mg tutin/kg and 6.0 mg hyenanchin/kg of honey. Ninety-five per cent both the tutin and hyenanchin was recovered.

The conservative reporting levels are 2.0 mg tutin/kg and 1.0 mg hyenanchin/kg.

RESULTS

The results of the LCMS analysis of the 20 honey samples are presented in Table 1.

Table 1. Hyenanchin and tutin levels in honey samples from eastern Bay of Plenty, Coromandel Peninsula and Waikato in 2008.

FBC Ref	Region	Client Sample Ref	Hyenanchin (mg/kg)	Tutin (mg/kg)
8238		Submitter A Honey 1	t	n.d.
8239		Submitter A Honey 2	1.5	n.d.
8240		Submitter A Honey 3	t	n.d.
8241		Submitter A Honey 4	t	n.d.
8242		Submitter A Honey 5	t	n.d.
8244		Submitter B Honey 1	n.d.	n.d.
8245		Submitter B Honey 2	t	n.d.
8250		Submitter B Honey 7	t	n.d.
8251		Submitter B Honey comb	2.6	n.d.
8252		Submitter C Honey 1	n.d.	n.d.
8253		Submitter C Honey 5	t	n.d.
8254		Submitter C Honey 10	n.d.	n.d.
8257		Submitter D Honey 803	n.d.	n.d.
8259		Submitter D Honey 844	n.d.	n.d.
8261		Submitter D Honey 886	n.d.	n.d.
8301		Submitter E Honey 1	t	n.d.
8302		Submitter E Honey 2	t	n.d.
8303		Submitter E Honey 3	1.7	n.d.
8304		Submitter E Honey 4	n.d.	n.d.
8305		Submitter E Honey 5	2.4	n.d.

n.d. = not detected
t = trace (the analyte positively found at a level below the quantified limit of detection)
A copy of the laboratory report may be found in Appendix 2.

DISCUSSION

The data show that in the 2007–2008 honey harvest season, in four out of five of the different regions sampled, honey can contain the tutu-related hyenanchin toxin. While no tutin was reported in these 20 samples, some significant levels of hyenanchin were reported. This shows the very real risk that tutin toxic honey can present – a fact borne out by the March 2008 outbreak of toxic honey poisoning in the Coromandel area.

The method of analysis is more reliable and sensitive than earlier methods using HPLC. It is felt that with further development the limit of detection is likely to be reduced from 2 mg/kg to the region of 0.2 mg/kg. This lower limit of detection is desirable so that the method may be used whenever honey needs to be assessed. The high recovery percentage (95%) of the spiked honey provides assurance of the method's robustness.

The full understanding of the risk of toxic honey to humans requires; a true toxicity measure, a known relationship between the test animal and humans, a reliable measure of the amounts of each toxin present and a known rate of sorbtion. The LCMS methodology developed at HortResearch, Ruakura together with the LD_{50} tutin toxicity are sound steps towards quantifying the toxicity of tutin honey.

CONCLUSIONS

This report presents new information on the threat that toxic tutu honey poses to the local beekeeping industry. The new tutin LD_{50} value is an important step in quantifying the toxicity of honey samples. In the report by Dr Rex Munday, he points out the uncertain estimate of the toxicity of the tutin metabolite, hyenanchin and also the possibility of other tutin-related toxins that may be present. These may be isomers of the known tutin and hyenanchin or other compound. The results of the 20 'at-risk' honey samples show that there can be toxic honey produced and indicate that a more extensive screen would be required to understand the extent of the potential toxic honey production.

The LCMS methodology developed at HortResearch, Ruakura together with the LD_{50} tutin toxicity are sound steps towards quantifying the toxicity. A wider understanding of the tutu toxic honey conundrum requires; a validation of the relationship between test animal and human toxicity, a known LD_{50} for all the toxic compounds in honey together with known toxic sorbtion rates. Increasing amounts of honey are being used in the cosmetic and pharmaceutical industries. The dermal sorbtion rate is considered to be slower than gut sorbtion, however, a longer exposure interval could bring the dermal toxicity levels to a level for concern equal to that of oral exposure.

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APPENDIX 1

TUTIN TOXICOLOGY REPORT: APPROACHES TOWARD RISK ASSESSMENT OF THE HONEY CONTAMINANT, TUTIN

by Rex Munday, AgResearch, Ruakura Research Centre. April 2008

INTRODUCTION.

The genus *Coriaria* comprises about 30 species of plants, found in Europe, Asia, South and Central America, the Pacific Islands and New Zealand. Many species of *Coriaria* are toxic, and deaths have occurred through eating berries or leaves of these plants (1-6). In New Zealand, 8 Coriaria species have been recognised (7), collectively known as 'tutu". Ingestion of tutu by farm animals may be fatal, and it was reported that settlers in the 1860s lost a significant proportion of their stock to tutu poisoning (1,8). In 1863, Riban (9) purified a toxic compound from the European *Coriaria myrtifolia*, which he named "coriamyrtin". In 1900, a compound with similar toxicity and chemical reactivity was isolated from 3 New Zealand species of *Coriaria* by Easterfield and Aston (10), which they named "tutin". The structure of tutin (Fig. 1) was established in 1963 (11).

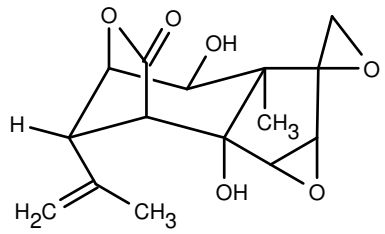

Fig. 1. Tutin

Land clearances, leading to destruction of tutu in agricultural areas, resulted in a decrease in problems with farm animals in New Zealand. However, a second problem, which was later shown to involve tutu, emerged in the early 1900s, when sporadic outbreaks of poisoning by honey were recorded (12). Initial symptoms included giddiness, abdominal pain and vomiting, followed, in some cases, by rigidity of the limbs and seizures. In severe cases, death ensued, and loss of memory was reported in survivors who had suffered severe seizures (1,2,12,13). Several plants were suspected as sources of poisonous nectar or pollen, but after a serious outbreak of honey poisoning in 1945, the origin of the toxin was identified as honeydew excreted by the passion-vine hopper feeding on the sap of tutu plants (14). Chemical studies on poisonous honey revealed the presence of not only tutin, but also hyenanchin (8-hydroxytutin, Figure 2, previously designated "mellitoxin"), which is formed by metabolism of tutin by the vine hoppers.

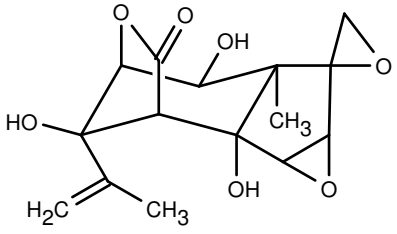

Fig 2. Hyenanchin

In response to outbreaks of poisoning, beekeeping in areas of the Coromandel Peninsula, Eastern Bay of Plenty and the Marlborough Sounds was restricted by law. The restricted area permits scheme was, however, replaced in 1991 by regulations requiring beekeepers to minimise the risk of contamination of honey with tutin or hyenanchin by removing hives from danger areas before the risk period (late December to the end of April) or by ensuring that tutu was not present in the areas foraged by bees around the apiary (15).

Much work on the chemistry of tutin and hyenanchin was conducted in the 1950s and 1960s, but no systematic risk assessment of these honey contaminants was conducted. Despite the regulations, cases of honey poisoning still occasionally occur. In view of the severe effects of tutin and hyenanchin on human health, and the potential impact of toxic honey on the industry, particularly in export markets, it is important that a detailed risk assessment is undertaken. In this way, the levels of the toxins in honey that are expected to have no adverse effects on human health can be identified.

Risk may be defined as "The probability that an adverse effect will be induced in an individual through contact with a particular substance". Risk is dependent upon two major factors – the inherent property of the substance to cause harm, which is generally assessed by toxicological studies in animals, and the amount of that substance to which consumers are exposed, which is derived from the amount of the toxin in the foodstuff and the amount of that foodstuff ingested by humans.

Several methods of analysis for tutin and hyenanchin, of varying sensitivity and precision, are reported in the literature. More recently, a precise and reproducible LC-MS method for these toxins has been developed, which will be invaluable in risk assessment. Some data on the acute toxicity of these substances are also available, as summarised in Tables 1 and 2. Unfortunately, previously-reported toxicological experiments were largely directed toward the development of a bioassay (12,16,17) and are neither of a standard now expected by regulatory authorities nor appropriate for risk assessment. The majority of the available data is concerned with the toxicity of tutin by injection. For risk assessment, it is important to administer the test material by the same route in which is taken up by the consumer, which, in the case of honey, is by ingestion. Furthermore, in the few reports involving oral administration, the data are inadequate, since the method of determining acute toxicity is not given, and imprecise parameters such as the "minimum lethal dose" have been used. Furthermore, the strain, sex, age and state of alimentation of the experimental animals were not recorded, and no attempt was made to establish a no-observable adverse effect level (NOAEL).

In the present report, the determination of the acute oral toxicity of tutin by an internationally-approved technique is reported, together with data on the effect of the state of alimentation on acute toxicity. Preliminary information on the NOAEL in mice is also presented.

MATERIALS AND METHODS

Pure tutin was provided by Don McNaughton (HortResearch, Hamilton) from a sample isolated by the late Dr. E. P. White. Solutions for dosing were made in water immediately before use.

Acute toxicities were determined according to OECD Guideline 425 (18), and LD_{50} values and confidence intervals were calculated with the statistical programme accompanying this guideline.

Tutin was administered at various dose levels by gavage to female Swiss albino mice, 6-7 weeks old, of initial body weight 18-22 g. Aliquots of the tutin solution were diluted to a volume of 200 µl and administered by gavage. In one experiment ("fed mice"), the animals were allowed free access to food at all times. In a second experiment ("fasted mice") food was withdrawn at 4 p.m. on the day before dosing, and replaced 10 minutes after administration of the tutin solution.

Mice were observed closely during the day of dosing. Survivors were observed and weighed each day for 7 days after dosing. Mice dying during the experiment and those killed at its termination were necropsied.

In experiments directed toward an estimate of the NOAEL, mice were dosed with tutin at levels below the LD_{50}, and their subsequent behaviour was monitored closely, comparing this with the behaviour of control mice dosed with water. Three mice were employed at each dose-level, set at 2.0, 1.0, 0.5 and 0.25 mg/kg. Fed mice were employed in these experiments.

RESULTS

1. Acute toxicity.

In fed mice, the LD_{50} of tutin was 4.7 mg/kg, with 95% confidence limits between 3.6 and 6.8 mg/kg. At lethal doses, abdominal breathing was noted within 5 minutes of dosing, and the mice were lethargic. After 15 minutes, the hind legs of the mice were slightly extended, and the animals showed little inclination to move. After 15-30 minutes, intention tremors were noted when the mice moved. These were first apparent in the head, but later progressed to the whole body. The hind legs of the animals were stiff. Tremors were subsequently observed in some animals even when at rest. After between 40 minutes and 2 hours, the mice fell on their side and exhibited rapid running movements for a few seconds. The hind legs then became fully extended and rigid, and the mice died. No macroscopic lesions were observed at necropsy.

At sub-lethal doses, hind leg extension and intention tremors were again observed soon after dosing. These persisted for 2-3 hours, when the mice were hunched and lethargic. Subsequently, the animals became more alert and moved without tremoring, although stiffness in the hind legs persisted, and they were less active than control mice. Their appearance and behaviour normalised at between 4 and 5 hours after dosing. They remained apparently normal throughout the subsequent one-week observation period, and no abnormalities were observed at necropsy.

In fasted mice, the LD_{50} of tutin was 3.2 mg/kg, with 95% confidence limits between 2.4 and 4.6 mg/kg. The symptoms of poisoning were the same as those in fed mice, although the

onset of such symptoms was more rapid. At necropsy, small haemorrhages were observed in the glandular stomach of two out of the three mice receiving fatal doses of tutin. No other macroscopic abnormalities were observed in fasted mice receiving tutin.

2. Behavioural changes in mice given low doses of tutin.

At an oral dose of 2 mg/kg, tutin induced obvious toxic effects, with abdominal breathing, intention tremors and hind limb rigidity. After 1 hour, mice were less active than controls. They were hunched, with slight piloerection. Their condition improved, however, and by 2 hours after dosing, they were indistinguishable from control animals.

At 1 mg/kg, abdominal breathing, hunching and hind leg stiffness were again recorded, and the mice were lethargic. Their appearance and behaviour normalised 1-1.5 hours after dosing.

At 0.5 mg/kg, slight hunching was observed, and the mice were less active than control animals. The effects wore off quite rapidly, and the dosed mice were indistinguishable from control animals in less than an hour.

At 0.25 mg/kg, no effects were observed.

DISCUSSION

Acute toxicity of tutin in mice.

The acute oral toxicity of tutin in mice was of the same order of magnitude as that observed in other species, although mice were more sensitive to tutin than rats, and less sensitive than guinea pigs. The symptoms of intoxication in mice were those of a neurotoxin, again consistent with earlier reports (1,17,19-22). Mice given sub-lethal doses of the toxin showed toxic effects, but subsequently recovered. They showed no delayed toxicity and no long-term consequences, in accord with reports in guinea pigs and rabbits (19).

Fasting is known to increase the acute oral toxicity of several toxins, an effect that is attributed to a decreased amount of food in the stomach of the fasted mouse, permitting more rapid transport of the toxin to the absorptive areas of the small intestine. Tutin was 1.5 times more toxic to fasted mice than to fed. It is interesting to note that in some fasted mice, haemorrhagic lesions were observed in the glandular stomach after fatal doses of tutin. Damage to the stomach mucosa will facilitate absorption from this organ, and this effect may therefore contribute to the observed increase in toxicity. Tutin appears to be an irritant to the stomach, since submucosal haemorrhage was recorded in the stomach of a pig poisoned with tutin (10) and toxic honey, administered by gavage, produced inflammation of the gastric mucosa in rats (13).

Signs of toxicity were observed in fed mice dosed with tutin at levels around one-tenth of the LD_{50}. The NOAEL of tutin in these animals was 0.25 mg/kg.

Comparative toxicity of tutin and hyenanchin.

Since toxic honey contains both tutin and hyenanchin, it is necessary to take account of both substances when conducting a risk assessment. From published reports, there appears to be

no doubt that hyenanchin is less toxic than tutin, but the ratio between the toxicities has not been unequivocally established. It is stated in the literature that hyenanchin is 5 times less toxic than tutin (23) or 9-10 times less toxic (13,17,24). Comparison of the acute toxicity data in Tables 1 and 2 shows that, by injection, the ratio is between 6 and 15. By oral administration in the guinea pig, the ratio is at least 10 (the toxicity data for tutin in the guinea pig was an LD_{75}, while that for hyenanchin was an LD_{50}). For the rat, the ratio was between 2 and 4.5, but it should be noted that these data are described as "approximate" (22). In experiments in which the mouse bioassay was used for the determination of toxicity, it appears that ratio of 9 was employed in order to express total toxicity in terms of "tutin equivalents" (25). An accurate value for the oral LD_{50} of hyenanchin is urgently required for risk assessment of honey samples, which invariably contain both tutin and hyenanchin.

Risk assessment - general principles.

In the risk assessment of acute effects, i.e. harmful effects that occur soon after consumption of the foodstuff, the appropriate parameter to employ is the Acute Reference Dose (ARfD). The ARfD is defined by the World Health Organisation as "An estimate of the amount of a substance in food and/or drinking water, normally expressed on a milligram per kilogram body weight basis, that can be ingested in a period of 24 hours or less without appreciable health risk to the consumer, on the basis of all known facts at the time of evaluation".

The ARfD is determined from toxicological studies in animals or from data from accidental poisoning in humans. The two parameters that may be employed for determination of the ARfD are the NOAEL or the Lowest Observable Adverse Effect Level (LOAEL). The ARfD is calculated from these by applying a suitable safety factor.

In risk assessment, it is important to select the appropriate safety factor in calculating the ARfD. Usually, a factor of 100 is used with animal data. This is made up of a factor of 10 to allow for extrapolation from animal to human, and a factor of 10 to allow for variations in susceptibility among individuals within the human population. For data derived from human experience, a factor of 10 (allowing for individual variation) is generally employed. Higher or lower factors may be judged appropriate by the toxicologist, however, depending upon the quality of the data available and whether the NOAEL or LOAEL is employed. The total amount of toxin that is predicted to be without harm when acutely ingested by a human is calculated by multiplying the ARfD by the body weight of the individual.

The "regulatory limit" or "guidance level" of a toxin in food (the concentration of the toxin in the foodstuff that is unlikely to be associated with appreciable health risk to the consumer) can be calculated from the above amount of toxin and the amount of the food consumed. Consumption of particular foods vary widely among individuals, so a value that would cover the majority (>98%) of the population is needed.

Risk assessment based on mouse studies.

The NOAEL for tutin in fed mice was 0.25 mg/kg. From this, by application of a safety factor of 100, an ARfD of 0.15 mg can be calculated for a 60 kg man. It could be argued that since honey is often consumed for breakfast, a more appropriate figure would be that relating to the fasted mouse. No data on the NOAEL in fasted mice is presently available. The LD_{50} of tutin in fasted mice was 1.5 times higher than that in fed mice. If this ratio was carried

through to lower doses, the ARfD would be 0.1 mg. Definitive studies on fasted mice are required, however, to further investigate this point.

Risk assessment based on reports of human poisoning.

A statement to the effect that "toxic effects were recorded in an adult man after a tutin dose of 1 mg" is frequently repeated in the literature, attributed to various authors. In fact, this appears to be derived from papers by Easterfield & Aston published in 1900 (10) and 1901 (2). These authors isolated tutin from tutu, and on the basis of melting point, it would appear that the material that they isolated was pure tutin. In the 1900 paper, it was stated that "a small dose, estimated to be about 0.01 grain, caused sickness and incapacity for work extending over 24 hr in a full-grown man". 0.01 grain is equivalent to 0.648 mg, but in the subsequent paper by these authors, the amount administered was changed, without explanation, to 1 mg. The route of administration was not stated, but other data in the first paper refer to toxicity by the oral route, so it may be assumed that this was also the route of administration to the human.

In the 1920s, there was interest in the possible pharmacological use of tutin for relief of asthma and for treatment of "states of excitement occurring among the insane" (26,27). Macpherson, in experiments reported in 1920, dosed men and women in a mental hospital with 1.0-2.0 mg of tutin subcutaneously, with no toxic effect (26). No details regarding the purity of the tutin used in these experiments were given, but the tutin was provided as a solution by Dr. Aston, who used apparently pure tutin in his experiments in 1900-1901. In 1929, Corban described experiments upon himself, taking oral doses of between 1 and 3 milligrams of tutin. He noted mild hallucinations, decreased mental acuity and prolonged nausea, but did not report limb rigidity or seizures. He used the same solution as that employed by Macpherson 9 years earlier, and considered the possibility of decomposition of the tutin in storage. Injection of this material at a dose of 1.1 mg/kg, however, killed a cat in half an hour (28). Corban later administered 1-2 mg of tutin subcutaneously to hospital patents, with no effects except in one case, in which tremors in the knees and slight twitching of the fingers were observed (27). Muscular twitching was seen after 5 subcutaneous doses of 0.5 mg/kg, given at hourly intervals, and epileptiform seizures occurred in an individual after the third of 3 doses of 1 mg tutin, given over a 36-hour period (27). Corban noted that Easterfield & Aston had shown toxic effects of tutin at lower doses than those used in his experiments, and suggested the possibility that some individuals may be much more susceptible to tutin poisoning than others.

A sample of honey from Te Teko, which was responsible for inducing sickness and delirium in a 7-year old boy in 1949 (12) was analysed by the mouse bioassay, and shown to contain 40 mg/kg of tutin equivalents. A sample from Whitianga, causing poisoning in humans in 1979, contained 60 mg/kg tutin equivalents (16). It is impossible to estimate the amount of toxin ingested by humans eating these honeys, however, since no information on the amount of honey consumed is available. It would be most useful if data on honey intake in the recent (2008) poisonings by Coromandel honey are available, along with the analytical data on tutin and hyenanchin content.

It is regularly stated in the literature that the lethal dose of tutin for a human is 5 mg. This stems from a comment by Palmer-Jones et al. (23) that "It appears probable that 5 mg is lethal to a human". This figure is therefore speculative, and is not based on any scientific evidence.

Are children particularly at risk of tutin poisoning?

On the basis of body weight, it would obviously take less tutin to poison a child than an adult. Other factors being equal, only one quarter of the amount of tutin would be needed to cause harm to a 15 kg child than that needed for the same effect in a 60 kg adult. But the question arises as to whether children are intrinsically more susceptible to tutin than adults, as has been shown for other natural toxins (29,30). There are statements in the literature that young animals are more vulnerable to tutin than older individuals (1,12,31), although no systematic studies have been conducted. From case reports, it is difficult to assess the relative susceptibility of children and adults to tutin. 34 boys at an English boarding school were poisoned in 1923, as was a 7-year old at Te Tiko in 1949 (12), but whether the same honey was consumed by adults is not known. Experiments in mice of different ages would provide information on the likelihood of childhood vulnerability to tutin. If this proves to be the case, it would be necessary to increase the safety factor in the risk assessment.

Are toxins other than tutin and hyenanchin present in toxic honey?

In experiments on the extraction of hyenanchin from toxic honey, Sutherland and Palmer-Jones (32) noted the presence of a material that was toxic to guinea pigs but was not extracted by solvents that would have been expected to remove both tutin and hyenanchin. This work, which was published in 1947, has not been followed up. A re-examination of toxins in contaminated honey, using modern methods of analysis, is needed.

Is tutin a cumulative poison?

The observation that tutin caused epileptiform seizures in an individual only after the third of 3 doses of 1 mg tutin, given over a 36-hour period (27) raises the possibility that tutin may accumulate in the body. Little is known about the rate of elimination of this substance, and the possibility that effects could be greater after regular consumption of honey, rather than after a single exposure, requires investigation.

CONCLUSION

Although tutin has been deliberately administered to humans, data on the amounts causing toxic effects are conflicting. It would appear that milligram amounts of tutin can cause harm, although the available data are not sufficiently precise to be of significant value in risk assessment.

Tutin is very toxic when dosed to mice by gavage. It is more toxic to fasted mice than to fed. In fed mice, the NOAEL was 0.25 mg/kg, based on behavioural changes. Further work on the NOAEL in fasted mice is required, since this state of alimentation may be more relevant to the human situation. Acute toxicity studies on hyenanchin are needed in order to establish a toxicity equivalence factor for this compound. Studies in mice of different ages are also required, in order to give information on the possibility that children are more at risk of tutin poisoning than adults. Studies on the possibility that toxins other than tutin and hyenanchin may be present in toxic honey are needed, along with examination of the possibility that tutin may be a cumulative poison.

On the basis of the mouse data available at the present moment, an acute reference dose for humans of 0.10-0.15 mg of tutin equivalents would appear to be appropriate. For establishment of regulatory limits, information on the amount of honey that would cover consumption by the majority of the population is required.

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TABLE 1. PUBLISHED DATA ON THE ACUTE TOXICITY OF TUTIN

Species	Route of administration	Parameter	Acute toxicity (mg/kg)	Reference
Mouse	Intracerebral	LD_{50}	0.014	16
Mouse	Intracerebral	LD_{50}	0.01	17
Mouse	Subcutaneous	MLD	4.0	20
Mouse	Intraperitoneal	LD_{50}	3.0	21
Rat	Oral, gavage	LD_{50}	~20	22
Rat	Subcutaneous	LD_{50}	~4	22
Rat	Intraperitoneal	LD_{50}	~5	22
Guinea pig	Oral, gavage	LD_{75}	1.2	22
Guinea pig	Oral, gavage	MLD	>1.5	1
Guinea pig	Subcutaneous	LD_{75}	0.75	22
Guinea pig	Subcutaneous	MLD	2	19
Guinea pig	Intraperitoneal	LD_{50}	0.7	22
Rabbit	Subcutaneous	MLD	1.5	20
Rabbit	Subcutaneous	MLD	2.5	1
Rabbit	Subcutaneous	MLD	1.7	19
Rabbit	Intravenous	MLD	1.25	20
Rabbit	Oral, gavage	MLD	~ 6	1
Cat	Subcutaneous	MLD	~0.375	1
Cat	Oral, gavage	"Lethal dose"	~ 0.54	10
Pig	Oral, feeding	"Lethal dose"	~ 8	10

TABLE 2. PUBLISHED DATA ON THE ACUTE TOXICITY OF HYENANCHIN

Species	Route	Parameter	Acute toxicity (mg/kg)	Reference
Mouse	Intracerebral	LD_{50}	0.21	16
Mouse	Intracerebral	LD_{50}	0.09	17
Rat	Oral	LD_{50}	~ 40-90	22
Rat	Subcutaneous	LD_{50}	~30	22
Rat	Intraperitoneal	LD_{50}	30	22
Guinea pig	Oral	LD_{50}	12	22
Guinea pig	Subcutaneous	LD_{50}	9	22
Guinea pig	Intraperitoneal	LD_{50}	9	22

APPENDIX 2

FOOD & BIOLOGICAL CHEMISTRY LABORATORY RESIDUE REPORT MAY 2008

Analysis: Hyenanchin and Tutin

Received from: HortResearch
Private Bag 3123
Hamilton
Attn: Don McNaughton

Date Received: 17.03-2008 – 01.04.2008 *Date Analysed*: 05.05.2008

FBC Job No: 708, 709, 710, 711, 719

Storage: Fridge at 4°C

Analyst: Dwayne Jensen

Analytical method: A representative sub-sample was taken and dissolved in water. The mixture was worked up using liquid-liquid partitions. Analysis was by APCI-MS using a consecutive reaction monitoring method in the negative mode.

Limit of Detection: 2 mg/kg tutin; 1 mg/kg hyenanchin

SUMMARY OF RESULTS:

FBC Ref	Client Sample Ref	Hyenanchin (mg/kg)	Tutin (mg/kg)
See attached sheet			
Blank fortified @ 6.3 mg/kg Hyenanchin & 5.1 mg/kg Tutin		6.0 (95%)	4.8 (95%)

Results are not corrected for recovery
n.d. = no detectable residue.
t = trace below limit of quantitation

Date: 14.05.2008

Checked by: J.M. Cooney

N.B. The results shown in this report apply to the sample or samples as received. This report cannot be reproduced in part without the prior and express approval of HortResearch.

SUMMARY OF RESULTS:

FBC Ref	Client Sample Ref	Hyenanchin (mg/kg)	Tutin (mg/kg)
8238	Submitter A Honey 1	t	n.d.
8239	Submitter A Honey 2	1.5	n.d.
8240	Submitter A Honey 3	t	n.d.
8241	Submitter A Honey 4	t	n.d.
8242	Submitter A Honey 5	t	n.d.
8244	Submitter B Honey 1	n.d.	n.d.
8245	Submitter B Honey 2	t	n.d.
8250	Submitter B Honey 7	t	n.d.
8251	Submitter B Honey comb	2.6	n.d.
8252	Submitter C Honey 1	n.d.	n.d.
8253	Submitter C Honey 5	t	n.d.
8254	Submitter C Honey 10	n.d.	n.d.
8257	Submitter D Honey 803	n.d.	n.d.
8259	Submitter D Honey 844	n.d.	n.d.
8261	Submitter D Honey 886	n.d.	n.d.
8301	Submitter E Honey 1	t	n.d.
8302	Submitter E Honey 2	t	n.d.
8303	Submitter E Honey 3	1.7	n.d.
8304	Submitter E Honey 4	n.d.	n.d.
8305	Submitter E Honey 5	2.4	n.d.