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Diacetone alcohol, a dispersant solvent, contributes to acute toxicity of a fipronil-based insecticide in a passerine bird

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Abstract

Fipronil, a phenyl pyrazole pesticide, is aerially applied in eastern Australia to control locust outbreaks, usually as "Adonis 3UL Insecticide®" (BASF), an ultra low (UL) volume formulation containing 0.3% active pesticide. We tested the toxicities of technical-grade fipronil, the Adonis 3UL formulation and its components in zebra finch, a native bird at risk of exposure in locust control regions. We estimated oral-dose LD50 by the Up-and-Down method. Under laboratory conditions, we identified unexpectedly high toxicities due exclusively to diacetone alcohol (DAA), a solvent making up 12.5% of the Adonis 3UL formulation. In contrast, finches were asymptomatic when exposed to 0.3% technical grade fipronil dissolved in a minimum amount of acetone. Depending upon the behaviour and persistence of DAA under field conditions, this formulation of Adonis 3UL may pose a far greater threat to the health of small birds and possibly other vertebrates than expected for fipronil alone.

Keywords: Adonis 3UL; Fipronil; Diacetone alcohol; Toxicity; Zebra finch

1. Introduction

Many pesticide studies have demonstrated significant differences between the toxicities of formulation components and the active ingredient (Marc et al., 2001; Paul et al., 2005; Peixoto, 2005; Braconi et al., 2006; Skandrani et al., 2006). For example, the presence of surfactants increase the toxicity of Roundup® (Mann and Bidwell, 1999) and Arsenal 250NA® herbicides (Grisolia et al., 2004) while additives are known to increase the toxicity of five insecticide formulations: Dicarzol 200®, Lannate 20®, Pirimor G®, Kiros EV®, and Talstar® (Skandrani et al., 2006). These findings demonstrate the importance of including appropriate formulation blank controls when assessing pesticide formulation toxicity.

Adonis 3UL insecticide®, a commercial fipronil-based pesticide formulation registered for use in Australia, is used to control locusts in semi-arid and agricultural areas (APLC, 2007). There is no toxicological information specific to this formulation, which contains 0.3% fipronil (active ingredient, by mass) and 12.5% diacetone alcohol (DAA, by mass; BASF 2003). Furthermore, although DAA is a commonly used solvent listed as an "inert of unknown toxicity" by the Environmental Protection Agency in the United States (USEPA 2004), its toxicological effects on vertebrates have not been examined. Fipronil is an effective neurotoxin targeting gamma-amino-butyric acid (GABA) receptors (Hainzl and Casida, 1996), and, despite the abundance of these receptors in vertebrate brains, there is still little available information regarding toxicological effects of fipronil in vertebrates. Available information however, demonstrates there is high species-specific variability in fipronil sensitivity across the few avian species studied; fipronil is highly toxic to the two galliform species tested, yet considered non-toxic to the Mallard duck (USEPA, 1996). This variability makes it extremely difficult to predict the toxicity of fipronil on unstudied species.

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In the course of evaluating the sensitivity of fipronil in zebra finches (*Taeniopygia guttata*), a native Australian bird species, we identified the toxicity of the commercial Adonis 3UL formulation to be greater than that of formulations using technical-grade fipronil (Kitulagodage et al., unpublished data). Consequently, the objective of this study was to examine the toxicity of Adonis 3UL and its ingredients to explain this discrepancy. Acute toxicity studies were performed using Adonis 3UL, technical-grade fipronil, a lab-made formulation mimicking the Adonis 3UL mixture, and DAA.

2. Materials and methods

2.1. Chemicals

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$ or (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulphinylpyrazole-3-carbonitrile), CAS no. 120068-37-3, 97% purity was obtained from Chem Services, Inc., USA (Cat. no. PS-2136). Adonis 3UL Insecticide® was obtained from BASF Australia Ltd. and contained 0.3% fipronil, 12.5% DAA in the carrier canola oil. Diacetone alcohol (DAA; $C_6H_{12}O_2$ or 4-hydroxy-4-methyl-2-pentanone), CAS no. 123-42-2, purity 99% was obtained from Sigma-Aldrich Pty. Ltd., USA (Cat. no. H41544).

2.2. Experimental animals

Adult male zebra finches were obtained from a breeding colony at the University of Wollongong. Birds were housed two per cage (38 × 44 × 34 cm) for at least 14 days prior to treatment to allow environmental adjustment. Commercial finch seed mix, water and grit were provided *ad libitum*.

2.3. Preparation of test substances

We prepared a lab version of the Adonis 3UL fipronil formulation, "Lab-Adonis", containing 0.3% technical grade fipronil and 12.5% DAA (15 mg fipronil dissolved in 625 mg DAA) prepared to a total volume of 5 ml using canola oil (the carrier used in Adonis 3UL). A 0.3% fipronil formulation, "Fipronil stock", was prepared using 15 mg technical-grade fipronil dissolved in 60 µl of acetone, then prepared to a total volume of 5 ml using canola oil. Acetone was chosen as the solvent due to the high solubility of fipronil in acetone (545.9 g/l; BASF 2005). A 12.5% DAA formulation, "DAA stock", was prepared using 125 mg DAA prepared in 1 ml of canola oil. In addition, we used fresh commercial Adonis 3UL and a canola oil control. The composition of substances tested is summarised in Table 1.

2.4. Up-and-Down estimation of LD50

Acute oral toxicity test procedures employed in this study follow those outlined in the Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals (OECD, 2003). This protocol was introduced in 1998 to minimise the number of animals required to estimate acute oral toxicity to a chemical. In summary, the first animal is administered an estimated sublethal dose as determined from available literature (175 mg/kg is the recommended default starting dose); if this animal survives, the dose administered to the next animal is increased by a factor identified from the OECD dose progression table (default 3.2; OECD, 2003); if it dies, the dose is decreased by the same factor. Birds were tested individually and observed routinely, during the immediate 48 h after dose administration. The "stopping criteria" to determine when a confidence range has been reached is as follows: if three animals survive at the highest dose in the progression, the chemical is considered to be of low acute toxicity hazard to the species; if five reversals are observed in any six consecutive animals tested (a reversal is a change in survival outcome) then a "confidence interval" of lethality between those two dosing concentrations has been reached. From this established confidence interval, the estimated LD50 (*e*LD50) value was calculated statistically using the maximum likelihood method as per OECD guidelines (OECD, 2003). This *e*LD50 allows for the substance to be ranked and classified according to the Globally Harmonised System for the classification of chemicals which cause acute toxicity (OECD, 1998).

Table 1
Proportion of fipronil and DAA per test substance

Test substance	Fipronil (%, w/v)	DAA (%, w/v)
Adonis 3UL	0.3	12.5
Lab-Adonis	0.3	12.5
DAA stock	0	12.5
Fipronil stock	0.3	0
Control	0	0

2.5. Test procedure

Birds were fasted overnight prior to testing and weighed on the day of dosing (average zebra finch weight of 13 g). Adonis 3UL, Lab-Adonis, the DAA stock, the fipronil stock, or the canola oil control were administered as a single oral dose via gavage. Dosing volumes followed OECD Up-and-Down guideline recommendations of maximum liquid dose volumes of 2 ml/100 g body weight (OECD, 2003). Food was returned 30 min after dosing and birds were video-recorded for the first 3 h following treatment and checked periodically over the next 48 h. Adonis 3UL was administered at progressive doses of 26, 37.5 and 55 mg fipronil/kg body weight (bw) based on a 3.2 factor according to the OECD dose progression schedule (OECD, 2003). To compare toxicity levels, doses of Adonis 3UL, the Lab-Adonis, the fipronil stock, the DAA stock, and the canola oil control were administered on a ml/kg bw basis at progressive doses of 8.7, 12.5 and 18.3 ml solution/kg bw (equivalent to 26, 37.5 and 55 mg fipronil/kg for solutions containing fipronil).

2.6. Vaporisation of DAA in test solutions

The vapour pressure of DAA is 0.97 mmHg at 20 °C and it has a slow relative evaporation rate of 0.14 (nBuAc = 1.0; Celanese 2000). To investigate whether significant amounts of DAA might be evaporated after aerial application, thus reducing likely avian exposure, we weighed 200 µl aliquots of each formulation (DAA alone, Lab-Adonis, Adonis 3UL, and canola oil) at 2-min intervals over 20 min. This was measured in triplicate under standard lab conditions with constant room temperature of 21 °C.

3. Results

3.1. Adonis 3UL toxicity

The *e*LD50 for technical-grade fipronil (OECD Up-Down method) was established in a previous study (Table 2; Kitulagodage et al., unpublished data). In the present study, a lethal dose confidence interval of fipronil in Adonis 3UL was established in five reversals (i.e., six individuals) and thus an *e*LD50 value of 45.41 mg fipronil/kg. This is equivalent to an *e*LD50 of 15.14 ml Adonis 3UL/kg (Table 2).

Table 2
Acute toxicity of Adonis 3UL and technical-grade fipronil in zebra finch

Toxicity units	*e*LD50	Confidence interval
Adonis 3UL	45.41 mg fipronil/kg 15.14 ml Adonis 3UL/kg	37.5–55 mg fipronil/kg[a] 12.5–18.3 ml Adonis 3UL/kg[a]
Technical fipronil	310.2 mg/kg	175–550 mg/kg[b]

[a]Present study.
[b]Kitulagodage et al. (unpublished data).

Table 3
Acute toxicities of test solutions administered to zebra finch

Test solution	*e*LD50 (ml/kg)	Confidence interval (ml/kg)	*n*
Adonis 3UL	15.14	12.5–18.3	7
Lab-Adonis	15.14	12.5–18.3	7
DAA stock	15.14	12.5–18.3	7
Fipronil stock	Non-toxic	Non-toxic	5
Control	Non-toxic	Non-toxic	5

3.2. Formulation component toxicity

Using the OECD methods, identical *e*LD50 values of 15.14 ml/kg were calculated for Adonis 3UL, Lab-Adonis and the DAA stock (Table 3). This was based on established identical toxicity confidence intervals, lying between 12.5 and 18.3 ml/kg. Both the fipronil stock and the control solutions were non-toxic to all birds at the doses administered (8.7, 12.5 and 18.3 ml/kg); despite the fipronil stock containing the same concentration of fipronil as Adonis 3UL and the Lab-Adonis solutions.

The comparative vaporisation test demonstrated all the test solutions had slow rates of evaporation. The total evaporative loss over 20 min as a percentage of initial mass was 0.4% ($\pm$0.02) for DAA, 1.4% for both canola oil ($\pm$0.02) and the DAA formulation ($\pm$0.04), and 2.3% ($\pm$0.03) for Adonis. The resulting evaporation rates were 2.0, 6.6 and 10.4 mg/h, respectively.

3.3. Signs of intoxication

Identical signs of intoxication were observed in all birds dosed with both lethal and sublethal amounts of Adonis 3UL, Lab-Adonis, and the DAA formulation. These included ataxia, wing drop, fanned tail feathers, diarrhoea and loss of righting reflex. Signs were observed as early as 1 min after treatment. When birds died, mortality occurred overnight with no specific posture on death. Of those birds that survived, recovery, based on return to normal activity, behaviour and feeding, was observed 24 h post-treatment. No signs of intoxication were observed in birds given the fipronil stock or the canola oil control.

4. Discussion

These results clearly demonstrate that the presence of DAA render fipronil formulations far more toxic to birds than would be predicted from evaluations of fipronil alone. This is shown by Adonis 3UL, Lab-Adonis and the DAA stock having identically toxic effects on zebra finches. By contrast, fipronil administered in a formulation lacking DAA (fipronil stock) was not toxic at the same administered doses. Therefore, the toxicity observed appears to be directly attributable to DAA exposure. The presence of DAA in the formulations is responsible for the seven-fold difference between the *e*LD50s in zebra finch for Adonis 3UL (45.41 mg fipronil/kg) and technical fipronil (310.2 mg fipronil/kg; Kitulagodage et al., unpublished data). Importantly, all test substances containing DAA provoked identical signs of intoxication in zebra finches, but very distinct to those observed in birds dosed with higher amounts of technical-grade fipronil without DAA (Kitulagodage et al., unpublished data). Fipronil acts by targeting gamma-aminobutyric acid (GABA) receptors, resulting in neural excitation and convulsions (Cole et al., 1993). Signs observed in zebra finches dosed with high amounts of technical-grade fipronil included involuntary wing flapping and convulsions; consistent with expected fipronil toxicity effects. Such indicators of fipronil toxicity were not observed in zebra finches in the current study, further demonstrating that DAA, not fipronil, was responsible for the observed toxicity in zebra finches dosed with our formulations.

Little is known about the ecotoxicity of DAA to terrestrial species, although it is known to have low toxicity to a few aquatic organisms tested (toxicity values greater than 100 mg/l; OECD 2000). To accurately assess the ecotoxicological impact of Adonis 3UL as a locust-control method, a better understanding of the behaviour of Adonis under field conditions is required. Considering that Adonis 3UL is applied as an aerial spray (APLC, 2007), the rate of vaporisation of the formulation and its components should be assessed. Available physical and chemical data (Celanese, 2000) and results of our simple comparative test, however, indicate that DAA has a slow evaporation rate. When released in air, DAA is expected to have a half-life between 10 and 30 days (JSL, 2005). Furthermore, about half of airborne DAA may accumulate in water, where it is stable indefinitely at pH 7 (OECD, 2000; JSL, 2005). With respect to the release of Adonis 3UL into the environment via aerial spraying, a significant proportion of the DAA released is likely to accumulate in surface water where it will remain as DAA for periods of weeks or months. In Australia, Adonis 3UL is used to control locusts in large areas of arid zone habitat (APLC, 2007). Although pesticide spraying in the vicinity of water bodies is strictly prohibited in operational guidelines for locust control, small pools of water, and subsequent rainfall cannot be easily avoided and these sources of water will be highly attractive to terrestrial animals in these arid areas. Thus,

the use of Adonis 3UL formulations with DAA represents a significant exposure risk for birds and other terrestrial vertebrates using water sources on or adjoining sprayed areas.

In addition to being an ingredient in the Adonis 3UL formulation, DAA has been used in an 8.5UL (8.5 g fipronil/l; personal communication; APLC, 2007) and a 10UL (10 g fipronil/l) Adonis formulation (Peveling and Demba, 2003) which are all ultra low volume (ULV) liquid formulations. ULV formulations are typically dispersed as fine droplet particles at very low application rates (Micronair, 2006), therefore reducing the amount of active ingredient and solution needed to achieve insect control compared to high volume methods (AFPM, 1999). There is no public information regarding the function of DAA in the Adonis formulation. However, since its most common use is in printing inks to achieve favourable flow and levelling characteristics (Celanese, 2000), it is likely that DAA is an effective dispersant in Adonis. Clearly, DAA in Adonis formulations should be replaced with an alternative solvent.

5. Conclusions

It is clear that DAA toxicity may pose a greater risk to birds than fipronil in relation to the Adonis 3UL formulation used in locust control. Care and due diligence are essential in developing new formulations. This study demonstrates that assessment of the active ingredient toxicity alone, in this case fipronil, is inadequate in evaluating the toxicity of pesticide formulations.

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