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Review Article

Toxic Consequences of Australia's Fire ant Eradication Program Pesticide Deployment- a Comprehensive Review

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Article Information

Received: September 05, 2025
Accepted: September 15, 2025
Published: September 20, 2025

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Citation: Rasmussen T, Lefringhausen A and Turni C. (2025) "Toxic Consequences of Australia's Fire ant Eradication Program Pesticide Deployment- a Comprehensive Review", 5(1); 10.61148/2994-0109/ARPB/084.

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Abstract:

In February 2001, a population of red fire ants (*Solenopsis invicta*) covering 12,367 ha was discovered in the Port of Brisbane, Australia, which prompted a massive eradication program which commenced in 2001. In 2023, the National Fire Ant Eradication Program (NFAEP) was extended for another 10 years, with the treatment area now covering 850,000 ha, comprising well over 15,000 known fire ant nests. In an attempt to eradicate the ants, baits are mixed with either pyriproxyfen or s-methoprene and distributed several times yearly by handheld spreaders or via helicopter regardless if ants are present or not. The NFAEP staff claim this level of pesticide use is safe and has no adverse consequences for the environment, citizens or their animals. This review examines the potential of eradication, explores the risks of toxicity to non-target invertebrate and vertebrate species from exposure to these broad-spectrum pesticides. Effects on soil and water including ecological impacts from potential biomagnification are also examined.
A range of effects were identified that collectively raise significant concerns about the safety of the current red fire ant eradication program pesticide.

Keywords: red fire ants, s-methoprene, pyriproxyfen, non-target species

Introduction:

In February 2001, the first population of red fire ants (*Solenopsis invicta*) was found in the Port of Brisbane, Australia in an area of 12,367 ha with 130 infested sites and 470 known colonies. It was estimated that the ants had been in Queensland for up to 15 years before discovery [1]. This prompted the establishment of the National Fire Ant Eradication Program (NFAEP), followed by a widespread application of pesticide over a large area where nests had been detected, and in adjacent buffer zones where no nests had been found. The eradication plan specified that near waterways, s-methoprene was to be sprayed four times a year, while in other places, pyriproxyfen was to be applied six times a year. Twenty-three years later in 2023, the NFAEP's eradication program covered an area of 600,000 ha and the fire ant nest count was over 15,000 nests [2]. In 2024 this area was increased again to 850,000 ha and included spraying of the original areas using a new 10-year extension of the previous program. This new program led to heavily resprayed areas, but nests still increased to 17,975 by the 7th of December 2024 (Figure 1).

Biodiversity impact justification for Australian eradication program

After the discovery of the first population of fire ants in 2001, a study to determine the impact of fire ants on biodiversity was initiated [3]. It is the only controlled investigation into the impact of the red fire ant on biodiversity in Southeast Queensland [1].

The study design was weakly powered based on biodiversity monitoring of one plot with fire ants compared to one in a similar environment without fire ants. The study reported increased biodiversity loss in the fire ant-infested plot. However, the study was never replicated, and it is unclear if the fire ant nest area had experienced prior pesticide application that may have affected non-target species proximal to the nest(s). The study was only semi-quantitative and may have been confounded by the non-infested control site being dominated by another foreign ant species, the African big-headed ant (*Pheidole megacephala*) [1], that is known to have significant detrimental effects on other invertebrates. This may have adversely impacted the comparison of effects from red fire ants on local diversity, as the control site had 79 other invertebrates compared to 45 other invertebrates at the red fire ant site, yet they concluded that the fire ant had an impact on other invertebrates [1]. Additionally, according to the Threatened Species Scientific Committee (TSSC) [4] due to the paucity of existing biodiversity research in 2001, it was considered impossible to determine whether the fire ant infestation had any impact on the biodiversity of threatened species. Despite these limitations the biodiversity study [3] has often been referenced as evidence for the necessity to perform the eradication biosecurity response with widespread pesticide use, such as the report by the Australian Government Department of Sustainability, Environment, Water, Population and Communities [1, 5], DAF via the NFAEP [6] and the Invasive Species Council [7].

Studies on the impact of invasive species are plagued with lack of data from before the invasion, which makes it difficult to decide if the difference in native diversity between invaded and uninvaded sites is actually due to the invasion [8]. Red Fire Ants (*S. invicta*) are not considered a superior competitor that suppresses native ants [8]. Greatly reducing the fire ants had no impact on the native ant species abundance [8]. Nonetheless, the NFAEP quantified the red fire ant risk as a significant impact on biodiversity, lending support to the broadscale spraying of pesticide on the declared fire ant zones in the South-east Queensland region.

Learnings from other control programs

The spread of red fire ants observed in Australia since 2001 aligns with observations of the Institute of Food and Agricultural Sciences (IFAS) Extension, University of Florida, who note that no control methods, except biological control agents, will permanently eliminate fire ants (UF/IFAS Extension) [9]. Attempts at control through use of aerial pesticide spraying in Florida, USA, have been unsuccessful. Unanticipated outcomes from such attempts included an increase in the fire ant population and detrimental effects on the environment, such as acute and chronic toxicity to non-target species [10], [11], [12] [13] and soil organisms [14] [15], affecting biodiversity [16] and bioaccumulations [17], biomagnification [18], resistance to pesticides [19] and degradation of the ecosystem [20]. One potential explanation for the exacerbation of the fire ant infestation in the USA was unintended harm caused by broad spectrum pesticides to non-target invertebrates including other ant species, which may have contributed to a loss of competitive inhibition against fire ant incursion. The fire ants are often the first species to reinvade treated areas, often in higher numbers than before treatment [21]. Research has clearly shown that certain native ant species can attack and eliminate red fire ant colonies defended by workers, particularly those ranging in size of 30 to 400 workers, thereby significantly influencing the survival of newly established fire ant colonies [22].

Florida researchers concluded that the reduction of native ant communities has repeatedly been shown to be a factor in hastening, not slowing, the invasion and as having the potential for other non-target impacts on aquatic and terrestrial arthropods and wildlife, which is not trivial. Red fire ants' dispersal pattern and behaviour appears to be entirely ignored by the NFAEP, as the ants thrive in early successional or disturbed habitats, such as roadsides and human habitats, with a preference for moist soils, be that naturally or irrigated [23],[24]. Eradications of invasive ant species worldwide have typically been achieved in infestation areas of less than 1 ha. The largest area of a successful eradication covered 1084 ha with 14 known colonies at Yarwun near Gladstone, where nests were injected with fipronil and a hydramethylnon-impregnated bait was applied in areas of heavy infestation. The NFAEP extended prophylactic treatments with Insect Growth Regulators (IGR) for more than one kilometer around the nests over 18 months. The nests were discovered in 2006, and in 2010 the area was declared pest-free [1] [25]. However, caution has to be applied as the statement of eradication came from the NFAEP [25] and was not validated by any other source. No eradication program has ever been successful in preventing fire ant establishment and their spread over larger areas and longer time spans unless the fire ant incursion was detected early [26]. Three incursions of fire ants into New Zealand, which all occurred at the ports of entry with only a few colonies, were successfully terminated [27]. The red fire ants were already in Australia 15 years before detection, in China at least 10 years before detection and in Taiwan more than five years. United States, Taiwan and China have lost the war against the fire ants, joined by more than 20 countries and territories [26]. Judging by the data showing Australia's spread of fire ants over the years from Queensland into New South Wales and up to North Queensland, with the latest detection of fire ants at a coal mine at Moranbah in central Queensland about 800 kilometers from a known site [27] the outcome is inevitable despite the 24 years of attempted eradication. The NFAEP's approach to treat a wide buffer zone where fire ants had not been detected, but were considered possibly present is contrary to the approach recommended by University of Florida's UF/IFAS Center for Land Use Efficiency which is "doing nothing where imported fire ants are not present or present in very low numbers and do not pose a problem". The best biological control method is considered to be the preservation of other ant species, as different ant species will compete for food and nesting sites and may also attack small fire ant colonies and kill newly mated queen ants [9]. The introduction or conservation of natural enemies of imported fire ants is another strategy used to control incursions [9]. In the southeastern states of America, a number of small hump-backed parasitic flies of the family *Phoridae* were released as biological controls in the fight against fire ants in 2011 [28]. Another *Phoridae* fly species recently discovered in Russia also shows potential as control in the fight against fire ants. The parasitoides scuttle fly (*Microselia rossica*) attacks Carpenter ants (*Camponotus vagus*) [29]. Not much research has been done into this family of ants and it is unknown if the genus *Microselia* is only preying on a particular ant species or is not species-specific [30].

Fire ant baits

The NFAEP bait is a granular mixture of corn and soybean oil

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treated with either a Fast-Acting Insecticide (FAI), which are sprayed onto nest directly, or Insect Growth Regulator (IGRs) which is distributed via helicopter or hand-held sprayers. The FAI comprises either indoxacarb (a neurotoxin [31]) or a mixture of hydramethylnon and pyriproxyfen. The IGRs used are s-methoprene ($C_{19}H_{34}O_3$) and pyriproxyfen ($C_{20}H_{19}NO_3$) [32]. The program applies the pyriproxyfen bait product to general land areas, with the s-methoprene bait product being used in areas near waterways and on organic farms [33].

Both s-methoprene (Isopropyl-(2E,4E,7S)-11-methoxy-3,7,11-trimethyl-2,4. – dodecadienoate) and pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine) are juvenile hormone analogues. They act as insect juvenile hormone agonists and prevent insects from moulting into adults [34]. Both growth regulators are broad-spectrum insecticides and are widely used to combat arthropods [35]. The large phylum Arthropoda accounts for around 80% of all animals in the world, with over 1.2 million species known [36], including insects (such as ants, aphids, bees, butterflies, cicadas, dragonflies, flies, and grasshoppers), arachnids (spiders, ticks, mites, scorpions), crustaceans (lobsters, prawns) and myriapods (millipedes, centipedes) [37].

Considered biological pesticides, pyriproxyfen and s-methoprene function by inhibiting reproduction or development of young, rather than through acute toxicity. However, research shows that certain species, such as crustaceans, are susceptible [38] [39]

The argument for the eradication of fire ants using widespread application of poison is the expected potential loss of biodiversity, yet there was no adequate research into effects on non-target species, the assumption being that the effect on biodiversity by the ants will far outweigh any negative effect of toxic bait.

In this review, we will examine the effects of spraying of either s-methoprene or pyriproxyfen baits over vast areas. We will consider the safety of such a massive campaign, given that this approach has not been successful in other countries and, judging from the increase of the infested area over 23 years of eradication attempts, did not perform any better in Australia. We will also look what impact the distributing of growth regulators over massive land areas has on biodiversity.

Area baited

After a 23 year eradication program using toxic bait in an area of 600,000 ha area consisting of two regions, about 13,000 red fire ant nests were found in 2023 [40]. According to the interactive map of the fire ant eradication program, the ants have since increased to 17,975 nests (Figure 1, depiction of interactive map on the 7th of November 2024) [2]. Following this the government extended the program for another 10 years, widening the treatment area to 850,000 ha [41], although fire ant nests had already spread outside of this zone [2]. This is a staggering expanse of land area to cover indiscriminately with a growth regulating chemical. For context, 850,000 hectares (8,500 km2), is a land area slightly smaller than Puerto Rico (9,104 km^2) or Lebanon (10,400km^2). In fact, the land area marked for treatment is larger than 34 (17%) of all the countries in the world and larger than 48 (87%) of all territories and dependencies [42].

The current NFAEP fire ant suppression and eradication scheme (2023-2027) targets the Queensland land area between Caboolture in the north to Tweed Heads in the south and stretches west to Gatton with repeated rounds of broad spectrum pesticide baits [43]. This land area has a significant agricultural output of several $100 million per year, including fruits, vegetables, dairy, beef and nuts, with most of the produce supplying the local domestic market [44, 45]. Not only is it very fertile land for agriculture, but it also comprises pristine native lands within national forests and houses some of Australia's threatened vertebrates and endangered ecological habitats.

Fire ants have been found outside this current targeted area. Figure 1 showed the occurrence past Toowoomba. According to the fire ant program "fire ants are sometimes found outside the containment area". On their website NFAEP lists the most recent detections in Moranbah in the Isaac Region (700 km from the containment area in Figure 1), Rathdowney in the Scenic Rim and Baringa, Forest Glen and Palmview on the Sunshine Coast [46] (Figure 2 [47]). Fire ants have also been found in Tweed Heads in New South Wales near the Queensland border and a pallet from the biosecurity zone in Queensland containing fire ants was intercepted in Perth, Western Australia [48].

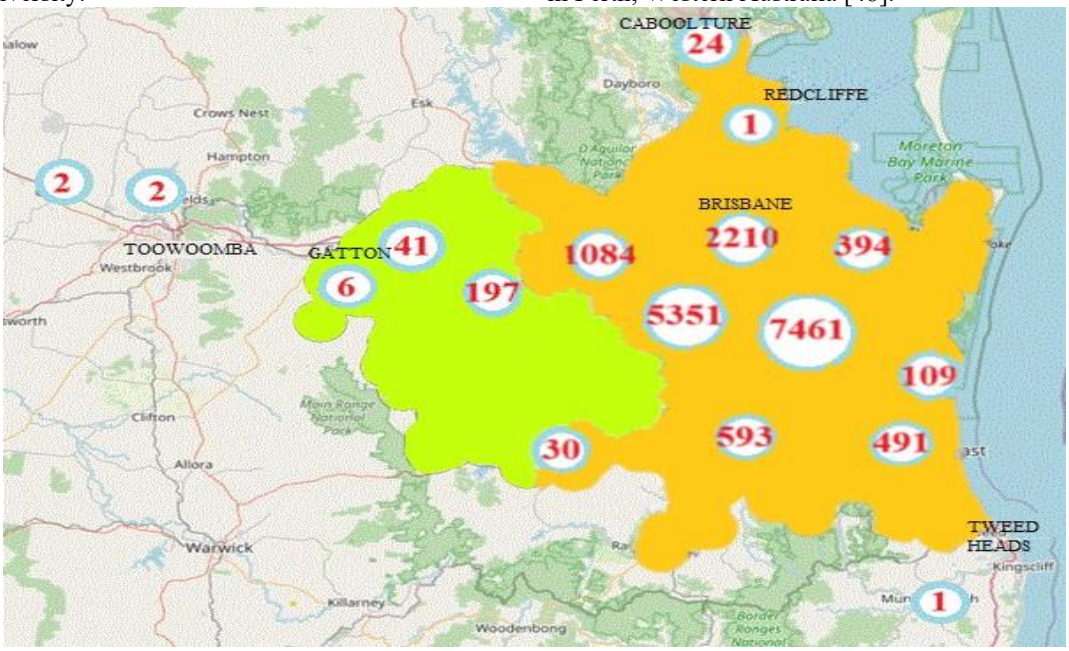

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Figure 1: Fire ant nests in South East Queensland. Contained within the orange and green area are the original zones of pesticide application in the 23-year period (2001 – 2023), which initially covered an area of 600,000 ha. The orange and green zones depicted here are the widened zones of fire ant eradications. This picture is a drawing of the NFAEP's interactive map [2] that showed the current fire ant spread as of the 7th of November 2024 and includes the current pesticide application area after a 10-year extension of the previous program. This new program has heavily reapplied pesticide to historic areas, and the nest count has increased to 17,975 [2].

Figure 2: A screen shot of the fire ant distribution according to the NSW government. © State of New South Wales. For current information go to www.nsw.gov.au. Licensed under the Creative Commons Attribution 4.0 license [47].

Persistence in the environment

According to the NFAEP, the baits degrade very quickly, which does not seem to correlate with real life studies done. Looking at the degradation of the active ingredients of the fire ant baits, a study by Webb and Jovic (2019) [49] found that after 8.5 days under 100% cover from the sun, neither pyriproxyfen nor s-methoprene showed any degradation of the active ingredients. Pyriproxyfen was much more stable losing only 10-20% of the active ingredients after 8.5 days under 50% cover and in full sun, respectively. S-methoprene was more sensitive to the sun and lost 91% to 75% of the active ingredients after 8.5 days in full sun and 50% cover, respectively.

Devillers (2020) [24] found that the half-life of pyriproxyfen in plants ranged from less than one week to about three weeks. In anaerobic aquatic environments, its half-life is 288.9 days [50]. That is, of course, not considering the toxicity of the degraded pyriproxyfen compounds.

According to PubChem, methoprene has a biodegradation half-life of approximately 10 days at a surface treatment rate of 1 kg/ha in sandy and silty loam soils [51].

Perception of Insect growth regulators (IGRs) safety from Mosquito control programs

Insect growth regulators (IGRs) are perceived as safe due to their history of prolific use against mosquitoes. Due to the significant

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detrimental impacts of mosquitoes on human and animal health, mosquito control programs are a well-established practice worldwide [52]. Both pyriproxyfen and s-methoprene are used in mosquito control, with s-methoprene used primarily in water environments. The assumption of IGR safety, however, while fortified by their widespread usage, lacks empirical evidence because long-term effects of IGRs spraying have not been extensively studied and particularly not when they were sprayed over large and diverse environmental areas. Only a few long-term studies exist, which cover small areas, and these studies report loss of diversity following IGR control programs. One study reported that spraying of s-methoprene on wetland at 0.05 – 0.058 kg a.i (active ingredient)/ha (the fire ant program sprays at 0.008 to 0.010 kg a.i/ha) over three years - six times during the spring and summer at 3-week intervals reduced insect density by 57 – 83% in year three, amounting to a biomass reduction of 50-83% during this test period [53] [54].

Methoprene is toxic to the many groups of insects and mites that exist in mosquito habitats, with members of the order Diptera being particularly sensitive [55]. Laboratory assessments of methoprene toxicity are usually compared with the levels used in mosquito control; it is generally accepted that areas which have undergone mosquito control have a final environmental level of 10 µg/kg in water. However, final concentrations range from 2-45 µg/kg when sprayed into water [55]. The fire ant bait (both s-methoprene and pyriproxyfen) is distributed at 5 g/kg using 1.6 -2 kg/ha (8 - 10 g/ha = 0.08 - 0.1 µg/ cm^2).

Despite the usage stipulations on the NFAEP permits for s-methoprene and pyriproxyfen, at least one case of contamination of a water body has occurred since 2023 when pyriproxyfen was sprayed into a creek in the Samford area, QLD. Local residents fundraised to have the creek water tested and pyriproxyfen contamination was determined to be 81 µg a.i./L, well above the pyriproxyfen safety level of potable water, which should not exceed 0.01 mg/L (10 µg a.i./L) [16]. In 2006, under its Pesticides Evaluation Scheme, the WHO recommended a larvicidal dose of 5–10 g a.i. (as granules)/ha (as granules) in non-potable water for controlling disease-carrying mosquitoes. The fire ant bait is sprayed at 8 - 10 g a.i./ha, resulting in 0.08 mg/L. Considering how significant the contamination of the all-year-round flowing creek in Samford turned out to be, questions about smaller water bodies, such as: "What is the concentration in a puddle of water or a little pond?" must be asked. These are sensitive habitats that support several of Australia's vanishing species, like e.g. where native frogs lay their eggs, and helicopters are actively spraying bait during the rainy season when frogs breed, while there is a lot of surface water around.

It is almost impossible to predict the modes and magnitude of dispersal throughout the environment and to non-target organisms. IGRs are soaked into corn grit and distributed in a solid form within a specific food type. Correlating the expected toxicity level posed by the bait to standardised scientific limits (such as Lethal Dose/Lethal Concentration) requires speculating the quantity of bait involved in every specific case; for example, the number of grits picked up and ingested by an organism, or the number absorbed into a water source. While researching how individual non-target species are impacted by IGRs, the authors discovered that most- if not all- of applicable research used for this literature review render toxicity data for pyriproxyfen and s-methoprene as ingested or absorbed into an organism in liquid form.

In some cases, a conversion of the bait's toxicity into its liquid equivalent is required in order to make some inference of the environmental impact resulting from the NFAEP baiting program. To address this variety of cases, the authors offer two approaches to calculate the likely dosages and concentrations being transferred to organisms and throughout the environment:

1. Direct conversion:

By weight, 0.5% of the corn bait used in the NFAEP program is IGR (pyriproxyfen or s-methoprene) [40]. To infer dosage to an organism via direct ingestion of the corn grit, simply multiply the percentage of IGR per grit with the weight of grit likely to be ingested.

When considering individual corn grit pieces, this approach also requires knowing the average size of a single grit. The commercial laboratory (Glorious Water Pty Ltd, Mount Glorious, Queensland) that conducted testing on behalf of the authors reported particle size of the grit to be between 0.5 – 1.5 mm. For the purposes of conversion, the authors propose considering the average size within this distribution as 1 mm.

An internet source provides the volume to weight conversion for corn grit at 673 kg/m^3 [56], which leads to a calculated weight of 0.000673 g/mm^3 for a 1 mm grit particle.

Based on these figures, the theoretical dose of IGR in one grit amounts to:

0.000673 x 0.005 = 0.000003365 g or 3.365 µg.

2. Dissolution into water:

A laboratory test was conducted on behalf of the authors to determine the concentration of IGR resulting from the dissolution of bait in water. This experiment served to determine the solubility of IGR from bait grit into water bodies such as ponds, rivers and creeks, as well as to infer dosage to an organism via ingestion or absorption of a contaminated water source.

For the test, a sample of 20 grits of NFAEP bait weighing a total of 0.028 g was soaked in 1L of water at 24°C for 24 hours. The resultant pyriproxyfen concentration was tested for and measured as 76 µg/L.

Given this result, it can be approximated that the dissolution of a single piece of bait grit in 1 L of water (76 µg/L/20) will produce a concentration of 3.8 µg/L of IGR.

It should be noted that this same calculation when done for grit weight (0.0014 g x 0,005 = 0.000007 g) would result in 7 µg/L of IGR. This could be due to solubility limits or a lower effective IGR content in the tested batch.

According to the fire ant program the baiting should yield an approximate 100 g/500 m^2 = 0.2 g/m^2 of total bait and 0.001 g/m^2 (1 mg/m^2) of IGR.

From the dissolution test, 0.028 g ÷ 20 = 0.0014 g/grit. Based on the density (1 mm^3), it's 0.000673 g/grit, which suggests bait size varies.

When calculating with a weight of 0.000673 g/grit, the result is 297.18 corn grits/m^2.

Using a weight of 0.0014 g/grit gives a result of 142.86 grits/m^2.

This variability of the bait size is shown in Picture 1. The baits particles were collected from a 1 m^2 plastic sheet spread out while a helicopter was distributing the bait (corn grit with pyriproxyfen and soybean oil).

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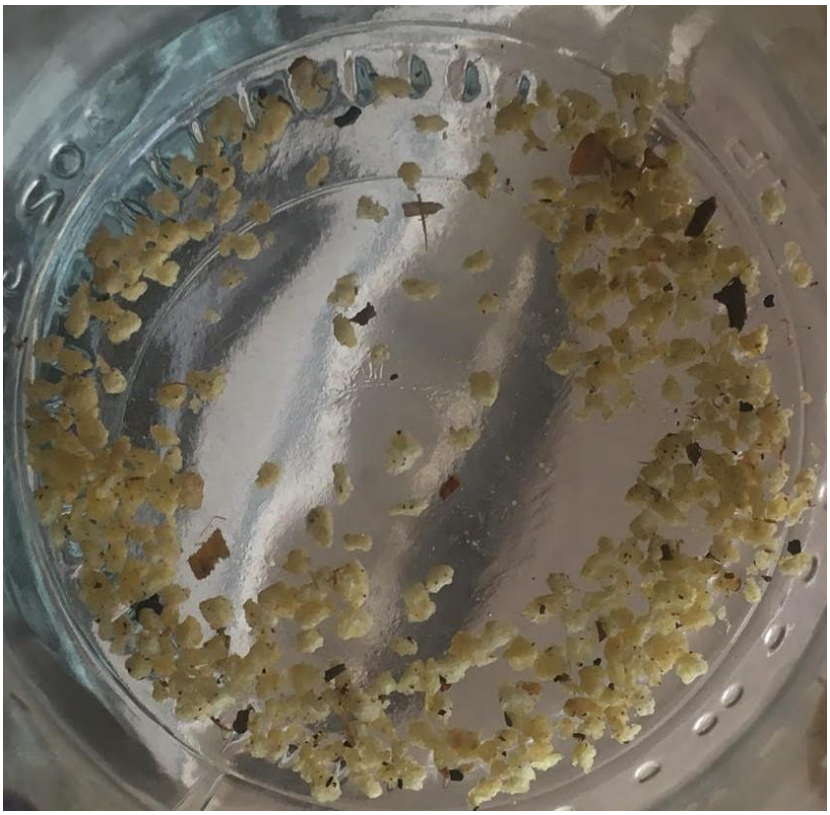

Picture 1 Corn grits of the pyriproxyfen bait collected on a 1 m^2 sheet while bait was distributed from a helicopter. Permission was given to use this picture.

The above approaches will be used intermittently in later sections of this paper to facilitate the application of published research on IGR toxicology to this case of broadscale bait distribution.

Pesticide resistance

So far, the issue of pesticide resistance has not been addressed. Studies in laboratories have shown that insects can quickly develop resistance to s-methoprene, which can result in cross-resistance to other pesticides [57, 58]. Spraying broad-spectrum pesticides over huge areas is not only affecting the environment now, but might be a significant problem in the future when pesticides lose their efficacy. Studies on mosquito spraying with s-methoprene report resistance ratios greater than 100 due to the methoprene pressure, which we have not seen before [57]. A study by Hopkinson et al. (2022) [110] tested the resistance level of pyriproxyfen against White Fly (Bemisia tabaci) in Australia, a significant agricultural pest primarily in cotton and soybeans. They found resistance ratios ranging from 4.1 to 56, with 18 of 69 populations from cotton resistant to 10 mg/L pyriproxyfen. At this concentration, the mortality ranges from 96 to 99.6 percent. However, viable nymphs were always present. In the laboratory strains, 1 mg/L of pyriproxyfen kills 100%; however, in field strains, the mortality only reached from 28.3 to 87.9%. When testing the population that survived 10 mg/L, two survived 30 mg/L, five populations survived 100 mg/L, and one population survived 300 mg/L. Pyriproxyfen has been used widely in the cotton and horticulture industries since the early 2000s. There were early reports of resistance beginning two years after use started. However, widespread resistance began to be reported in 2017 when the insecticide was used frequently due to constant problems with White Flies. Despite the low use by the cotton industry in the year of the study, numerous resistant populations were found.

Development of resistance to methoprene, which can occur in as few as eight generations, has also been discussed. Studies inducing resistance to methoprene have resulted in cross-resistance to other pesticides [55].

The NFAEP is and has been spraying pyriproxyfen over large areas six times a year for many years, which has the potential to lead to resistance of other agriculture pests and leave farmers with problems controlling their pests. Combined with the spraying likely to cause the loss of ant species that also control insect populations, this could lead to significant future problems for agriculture in Australia. The potential for resistance to be cross-reactive is not addressed either. Insects might become resistant to other insecticides as a reaction to the growth regulators.

Safety testing of fire ant bait over large areas

In 1995, the APVMA (at that time termed the National Registration Authority (NRA)) approved pyriproxyfen for use in Australia in the product 'Sumilarv insect growth regulator' for the control of cockroaches and fleas by licensed pest control operators. At this time, pyriproxyfen was not registered for use in other countries. The Public Release Summary for Sumilarv (pyriproxyfen) specified that Sumilarv was designated for use in 'household, commercial and industrial situations'. Considering the environmental fate of pyriproxyfen, the assessment asserted that the possibility of pyriproxyfen coming into contact with or becoming persistent in the environment or causing potential harm to native fauna and flora was highly unlikely due to its limitation to interior use. Moreover, the testing conducted for the assessment

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was also limited in scope due to the perceived limit on application. However, ecotoxicity was suggested to be high to very high for non-target insects and it was expected to be most likely highly toxic for fish [59] The NFAEP is now using this restricted product in large areas without any science to back the safety of spraying over 850,000 ha with growth regulators.

The NFAEP argues that it is safe for other insects as it has to be brought to the nest and consumed by the queen ant or eaten directly by other insects. However, many animals consume corn grit and soybean oil. Montgomery's et al (2022) [19] literature review pointed out that the granular bait based on corn grit and soybean oil quickly absorbs moisture from dew and rain, which reduces its oil content and makes it soggy, which in turn means the palatability is unpredictable. It is furthermore possible for the bait to break up due to moisture and subsequently release the insecticide. When the bait breaks down, the insecticide could be exposed to the environment, potentially making it more accessible to non-target organisms and it could potentially leach into soil and water affecting wildlife, plants and aquatic systems.

There is also the risk of contaminated water being taken up by animals. The bait is small enough to be consumed with other food by larger animals. According to the labels of these growth regulators, they can be taken up by consumption or accidental swallowing, skin contact and inhalation. Studies with pyriproxyfen have shown that bees do not have to take up the bait pellet; they only have to collect contaminated nectar sources, which results in the indirect transfer of pyriproxyfen to European honey bees [60] [61].

The risk of bioaccumulation has been hinted at in the literature [62]. Hence, larger animals just have to either consume the bait directly or via drinking water over a longer period, or consume animals that directly consumed the bait and stored the insecticide in their adipose tissues.

Soil

Fewer studies are available regarding the long-term impacts of pyriproxyfen and s-methoprene on soil, presumably due to their prior usage being limited to mosquito control and agriculture. Wetland areas, where mosquito control usually takes place are unlikely to be the site of soil biome analyses; the study of biota health within soil is also uncommon in conventional agriculture research, where ploughing and fertilisers are generally used to enhance soil nutrient content in place of a natural soil biome.

The European Union (EU)'s review for the approval of active substances (pyriproxyfen) in 2019 highlights this as a data gap, noting that 'Information to address the risk to sediment-dwelling organisms was not available, therefore a low risk could not be concluded for exposure via the sediment phase' [63]. The scope of use for pyriproxyfen in the EU review is for application on various agricultural plants; as such, it is reasonable to assume that the NFAEP's methodology of spreading pyriproxyfen in bait form over soil would be affected by the same gap in risk assessment.

Soil, which contains up to 59% of the world's diversity, is considered the world's most complex ecosystem [64]. It is well known that pesticides have detrimental effects on invertebrates that live in or develop as young in the soil, and which are fundamental for agricultural sustainability [17]. Excessive or indiscriminate application of pesticides can lead to environmental and health issues [14].

Studies show that the toxicity of pyriproxyfen's metabolites is far more significant than the toxicity of pyriproxyfen itself, and that these metabolites persist longer in the soil [65] [15] [13]. A study by Kumari et al. [14], which assessed the toxicity and persistence of pyriproxyfen and its metabolites in soil, found that metabolites A, B, C, E and F are suggested to be "very toxic", whereas metabolite D has been counted under the "extremely toxic" category [14]. All six metabolites were found to persist for more than 30 days in soil, and metabolites C, E and F caused toxicity to soil enzymes, such as sucrase, catalase, urease and dehydrogenase. There are serious concerns raised over the potential of these metabolites to cause toxicity through environmental contamination [14] [15].

Devillers (2020) [66] noted that while pyriproxyfen had a high Kow value (and was, therefore, a poor leacher), three of its metabolites had low Kow values and were found to be mobile in soil and water. One of these metabolites, PYPAC, was reported as having a high or very high mobility and the potential to contaminate groundwater [66].

Liu et al conducted studies on the effects of pyriproxyfen's metabolites on soil and found that as much as 120 days after exposure, most soil enzymes had not recovered to their original states [15]. The group also found that although pyriproxyfen was low in acute toxicity to adult earthworms, its metabolites were highly toxic, with the lethal concentration (LC50) for earthworms being an order of magnitude lower for all 6 metabolites compared to the parent pyriproxyfen [15].

A study by Chang et al. (2012) [67] examined changes in the soil bacterial community after application of either 1 mg/kg, 5 mg/kg or 10 mg/kg of pyriproyfen. They found that the number of bacterial species decreased with increasing pyriproxyfen concentrations, which translates to a lower diversity in the soil. It is recognised that soil microbiomes as a fundamental component of soil ecosystems are crucial for maintaining soil quality. A loss of diversity indicates a less healthy soil [68].

Using the thermophilic eubacterium *Bacillus stearothermophilus* as a model for determining the effect of methoprene on normal cell growth and viability it was found that the interaction of methoprene with the membrane and perturbation of cell bioenergetics might be the underlying mechanism of this compound's toxicity in non-target organisms [69]. *B. stearothermophilus* has previously been used as a model for toxicological evaluation of other environmental pollutants.

Waterways

Because Insect Growth Regulators as a class of insecticides are synthetic compounds that mimic the action of juvenile hormones by blocking the metamorphosis of insect larvae to reproductive adults [70], they are deemed to be safe. However, even the permit says that both growth regulators are very toxic to aquatic life. The permit stipulates "DO NOT contaminate wetlands or watercourses with this product or used containers." One example for instant toxicity after exposure to concentrations < 10 mg/L of pyriproxyfen is the high mortality for certain species' embryos, such as Zebra fish (*Danio rerio*) [71],[72],[73],[74], South American Claw fish (*Rhamdia quelen*) [75] and African clawed frog (*Xenopus laevis*) [76].

Table 1 summarizes adverse effects including acute toxicity of pyriproxyfen and s-methoprene for various organisms as sourced from available research publications.

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Table 1: The Effects of growth regulators on aquatic species are listed, including the adverse effects and concentration (Conc) at which the effect is observed.

S-methoprene/ pyriproxyfen	Animal species	Adverse effect	Conc	Reference
methoprene	shrimp	Reduced the shrimp's body length and molting frequency. bioaccumulation	100 µg/L	[143]
methoprene	lobster	Bioaccumulation in hepatopancreas, gonadal tissue, nervous tissue and epidermal cells	95-fold higher than environmental water levels	[144]
pyriproxyfen	*Daphnia carinata* neonates (< 12-h old)	48-h LC_{50} (lethal concentration 50%)	0.08 mg/L (0.06–0.11 mg/L)	[145]
	Daphnia carinata	The sex ratio changed; all the produced neonates were males	1 µg/L	[146]
pyriproxyfen	amphipod *Gammarus fossarum*	Inhibition of spermatozoon production by 40% at 5 µg/L to 73% at 50 µg/L	5 and 50 µg/L	[147]
pyriproxyfen	*Megacyclops viridis*	High mortality during the egg-hatching-nauplius stage	0.1 mg/L	[148]
pyriproxyfen	freshwater fish *Xiphophorus maculatus*	erratic swimming, loss of equilibrium, and lethargy after 24 and 72 h of exposure	5, 10, and 20 µg/L	[149]
pyriproxyfen	*X. maculatus*	ability to capture larvae significantly decreased	10 µg/L	[149]
pyriproxyfen	Embryos of zebrafish (*Danio rerio*)	pericardial edema and scoliosis, elongation of heart, yolk sac edema, and hyperemia	1.66 mg/L	[150]
pyriproxyfen	African clawed frog, *Xenopus laevis*	The ^{14}C concentrations gradually increased, almost reached the plateau after 7 days. The steady-state bioconcentration factors were calculated to be 550–560	3 µg/L	[151]
pyriproxyfen	Zebra fish (*Danio rerio*)	Lethal to embryos	< 10 mg/L	[71] [73] [74]
pyriproxyfen	zebrafish (*Danio rerio*)	Chronic low DFB exposure caused hypoactivity in anxiety and hyperactivity in social contexts, reduced anxiety-like behavior and aggressive interactions, and increased pro-inflammatory gene	0.379 and 0.758 mg/L pyriproxyfen for 30 days	[152]

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		expression but decreased *nfe2l2* gene expression.		
pyriproxyfen	zebrafish (*Danio rerio*)	Inhibited acetylcholinesterase activity and an increase in generation of oxygen and nitrogen-related species activity	treated for 16 hours with pyriproxyfen at concentrations of 0.001 mg/mL, 0.01 mg/mL, and 0.1 mg/mL	[153]
pyriproxyfen	zebrafish (*Danio rerio*) embryos/larvae	pericardial edema, scoliosis, elongation of the heart, yolk sac edema, hyperemia, and red blood cell accumulation	Concentrations of 0.16 µg/mL, 0.33 µg/mL, and 1.66 µg/mL	[154]
pyriproxyfen	zebrafish (*Danio rerio*)	Significant reduction of total length and body weight at the two highest concentrations	concentrations of 15.6 µg/L, 31.2 µg/L, 62.5 µg/L, 125 µg/L, and 250 µg/L	
pyriproxyfen	African clawed frog (*Xenopus laevis*)	Lethal to embryos	< 10 mg/L	[151]
pyriproxyfen	South American Claw fish (*Rhamdia quelen*)	Lethal to embryos	< 10 mg/L	[75]

Plants

Methoprene affects not only animals but also some plants. Several studies show that methoprene displays phytotoxicity on plant species, such as delayed female flower and plagiotropic bud appearance in spaghetti squash and coffee plants, respectively [77]. Looking at different plant species, the most severe adverse effects of Insect growth regulators were related to growth parameters of chickpeas and lentils [66].

Studies have shown that plants absorb pyriproxyfen; hence, animals eating plants potentially absorb the pesticide. The fire ant program involves spraying pyriproxyfen at 5 g/kg. There is no withholding period on the permit (Permit Number PER87728) but produce that is traded for human consumption and has direct contact with the bait must be washed after harvest and before marketing. However, if pyriproxyfen is sprayed onto plants, it behaves as a translaminar insecticide, moving across leaf tissues and affecting insects that come into contact with it. As pyriproxyfen moves into the plant tissue only a small portion remains on the surface or bound to the waxy cuticle, so that washing will only remove part of the insecticide. According to a publication by Devillers (2020) [66], the half-life of pyriproxyfen in plants ranged from less than one week to about three weeks, depending on the crop and the experimental conditions.

Another study concluded that excessive and uncontrolled usage of pyriproxyfen may result in phytotoxic effects due to the induction of morphological, anatomical, physiological and metabolic processes. As pyriproxyfen accumulates, it is thought to adversely affect all living beings feeding on plants that are exposed to it, as well as environmental factors such as soil, air and water [78].

Evidence for bioaccumulation

The current NFAEP program consists of repeated rounds (up to six times/year) of broad-scale spreading of pyriproxyfen and s-methoprene bait over the 850,000 ha zone [43].

This method of repeated applications of a low dose toxin opens the doors to potential bioaccumulation in both environment and non-target organisms. As observed in previous global bioaccumulation crises, like those involving mercury and DDT [79] [80], toxins can accumulate at higher concentrations with each step up the food chain, ultimately putting predator species and humans at risk.

Many studies show that pyriproxyfen and s-methoprene are contained in the tissues of animals after exposure. Liver and adipose tissue are the main organs for storage of pyriproxyfen metabolites with one of the twelve metabolites, 4'-OH-pyriproxyfen found most abundantly [62].

When carbon-14 (C-14) labelled methoprene was orally administered to rats, it was observed that after five days slightly less than 20% was excreted in the urine and a similar amount in faeces. Almost 40 % was exhaled. However, about 17% was retained in the body with 84.5 ppm in the liver, 29 ppm in the kidney, 26 ppm in the lung, 36.5 ppm in fat and 12-13 ppm in the adrenal cortex [81].

A single dose of methoprene was eliminated over 14 days from the eggs of laying hens, while C-14 labelled methoprene was detected

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in all tissues and organs examined [82]. In a previous study by Quistat (1975) [83] a single dose of C-14 labelled methoprene left residual radioactivity in both tissue and eggs.

The metabolic fate of methoprene was studied in a guinea pig, a steer and a cow. It was discovered that a large percentage of the radiolabeled methoprene was incorporated in the tissue and expired by the animals. A small amount was metabolised into free primary metabolites in urine and faeces [84].

When 25 milligrams per kilogram carbon C-14 labeled methoprene was given to Wistar rats orally, the highest tissue C 14 concentrations occurred 6 to 12 hours after dosing and were found in the liver, lungs, kidneys and fat [85].

Nimet et al (2021) [86] studied effects on adult America Bull Frogs (*Lithobates catesbeianus*). They exposed the frogs to different dilutions (0.002 g/L dilution (0.1 g/50 L) and 0.02 g/L) of pyriproxyfen (Sumilarv®) over 50 days. Adult frogs exposed to the standard dose recommended by the World Health Organization (WHO) (0.1 g/50 L) showed pyriproxyfen bioaccumulation in their adipose tissue in significantly higher concentration than that of control animals. The highest concentration of pyriproxyfen in the adipose tissue was observed in the frogs exposed to a 10 times higher concentration than recommended by WHO.

Bioaccumulation can cause chronic effects on the metabolism of animals. It can undergo biomagnification when these potentially toxic elements are inserted into the food chain, leading to adverse impacts on other organisms. Arthropods are an important mediator of pesticide transfer in the food chain, with sublethal doses critical for the biomagnification process [87], [88].

Non-target species affected

Consequently, this broad-spectrum insecticide could affect a diverse set of animal species, many of which serve as food sources for birds and other higher trophic level animals. Even if pyriproxyfen does not directly harm higher trophic species, such as amphibians, birds and mammals, it may affect them indirectly through the destruction of their food sources [89]. Pyriproxyfen is known for its high environmental stability and persistence in the food chain, leading to detrimental effects on non-target species [18].

Native ants

In a study by Webb (2014) [10], the comparative attractiveness of pyriproxyfen ant baits was tested, and it was found that five native Australian ant species were also attracted to the baits. In this paper, Webb states: "Over the past 10 years, various modifications to the standard corn and oil formulation in Distance® have been investigated to improve attractiveness for a wider range of ant species than just the original key target species, red imported fire ant." This indicates a clear risk of environmental damage to the distribution zone, as the 1,275 described Australian ant species are essential for the ecosystem's health. Ants aerate and turn over the soil, help in the dispersal of seed, help in the germination of seeds (some of our endangered plants cannot germinate without the help of ants), ants consume organic material and provide food for other organisms, control pest populations, and their presence is vital to ensure a balanced flow of energy in the ecosystems [90].

Invasion of ant species into Australia is nothing new and invasive ant species such as the red imported fire ant species do not seem to have an impact on native ant species [8]. However, baiting for invasive ant species leads to a decline of the abundance of non-target ants. One such example was the baiting of tropical fire ant, *Solenopsis geminata*, with hydramethylon on Spit Island, Midway Atoll, Hawaii [91]. A study by Webb et al (2013) [92] examined the effectiveness of Distance (5g/kg pyriproxyfen) to control meat ants (*Ididomyrmes sanguineus Forel*) in Western Australia by applying 5 g/mount on time, which is broadly analogous to the 2 kg/ha application. This baiting resulted in 85% reduction of mound activity by 51 days after treatment. The same reduction was seen for Engage (s-methoprene 5 g/kg, sprayed at 2 kg/ha application) [93].

Long term monitoring of effects of the Fire Ant Eradication Program on key local ant genera at some sites in Brisbane showed that ants of the genus *Pheidole* significantly reduced in abundance over time, suggesting that they were affected by treatment efforts [94].

It was already mentioned that other ant species play an important role in keeping fire ants under control. In Mississippi, USA it was found that the ant species *Paratrechina melanderi arenivaya* preyed on the fire ant *S. invicta*'s eggs, carrying the eggs to their own nest to be consumed by workers and larvae [93]. Australia, contrary to many other countries, has an ideal climate for ants that supports an abundant and diverse fauna of specialist predator ant species with one of the better known being bull ants of the genus *Myrmecia* [95].

In conclusion, the fire ant elimination program has a negative environmental impact by negatively affecting other ant species. Ants not only help with seed dispersal, pollination, soil aeration, and nutrient cycling, but they are also predators and help control the insect population, thereby maintaining the delicate ecological balance. Our native ants are also the prey of other species, such as the echidna, whose food source will be decimated by this program. It is reasonable to conclude that the elimination of native ant species will have enormous negative environmental impact. Ants, like frogs, have been used as bioindicators of environmental health and integrity [95], and it has been found that there is a correlation between the variety of ant species with diversity and/or abundance of a wide range of other invertebrate taxa [95]

Bees

Pyriproxyfen is highly stable in the environment and persists in the food chain, which leads to detrimental effects on non-target species [96]. Several studies have been conducted on the impacts of pyriproxyfen and s-methoprene on pollinators. Neither have been observed as being acutely toxic to adult bees when used at their normal dosage rates for crops and mosquito control. According to the Material Safety Data Sheet for s-methoprene (Sumitomo Chemical 2013), the LD_{50} (lethal dose 50%) for adult honey bees both orally and topically is 1000 µg a.i. (active ingredient)/bee [97]. The 48-h LD_{50} (lethal dose 50%) values for both oral and contact contamination for pyriproxyfen are greater than 100 µg a.i. (active ingredient)/bee which is why pyriproxyfen is regarded as having a low acute toxicity for adult honey bees [98].

However, these pesticides have pernicious and irreversible effects on bee populations, disrupting the development of bee larvae and affecting honey production, bee flight patterns and behaviour. In worker bee larvae, experimental studies showed abnormal formation of abdomen, wings and wax glands. Hormonal regulation in bees is disrupted by methoprene [99]. A study by Luo (2021) [15] showed that the environmental concentration (50 ng/µL) of pyriproxyfen significantly reduced the level of juvenile hormone in honey bee (*Apis mellifera*) worker larvae, a hormone

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playing a crucial role in their development. Prior research found that honey bee larvae do not survive if exposed to 100 ng/µL of pyriproxyfen.

The EFSA report (EFSA, 2019) determined the toxicity of pyriproxyfen for *Bombus terrestris* (Buff-tailed bumblebee or Large earth bumblebee) by oral or contact contaminations as >72.8 µg a.i./bee and >100 µg a.i./bee, respectively [98]. When topically applying 50 µL of a pyriproxyfen solution (25 mg a.i./L) on the thorax of *B. terrestris* worker bees, no adverse effects were observed; however, when this solution was sprayed onto pollen until saturation, significant larval mortality was observed [100]. When topically applying 1 µL of acetone with 14C-pyriproxyfen (15 mCi/g) 34% ± 3% penetrated the cuticle of the bees after 24 hours, explaining the low toxicity via topical pyriproxyfen exposure [100]. The EFSA (2019) concluded that a high risk to bumble bees cannot be excluded for the representative uses of pyriproxyfen on citrus, pome fruits and tomato (outdoor use). Screening assessments for solitary bees were also performed (acute adult, chronic adult and larva). Except for the acute risk for the representative use of citrus, these calculations also indicated that a high risk to solitary bees cannot be excluded for the representative use of citrus, pome fruits and tomatoes (outdoor use).

A study by Chang et al. (2015) [12] found that methoprene treatment accelerated the onset of both flight and foraging behaviour in worker bees, but it also reduced foraging span, the total time spent foraging and the number of completed foraging trips.

Studies are clear that bees would not have to ingest fire ant bait, as the collection of nectar from contaminated sources can result in the indirect transfer of pyriproxyfen [60] [61] [12]. Unfortunately, a study by Naiara Gomes et al. [101] also found that when a test tube with contaminated food at the field concentration (62.50 µL/L) was used to feed bees, only 10% of the bees avoided pyriproxyfen-contaminated diets. They concluded that this could indicate that bees will not avoid pollen and nectar contaminated by pyriproxyfen residues. This finding corresponds well with a 2012 study by Corrêa Fernandez et al. [102] who used the chronic larval test developed by Aupinel et al. [103] [104] [105] to investigate the effects of pyriproxyfen (1, 100 and 1000 ng/µL) on the flight muscle differentiation, vital for foraging and mating activities, in the Africanized *A. mellifera* honey bee. They found that for all concentrations the differentiation of the flight musculature was delayed compared to the control.

Most of our data about toxicity comes from the Apis bees (honey bees). There is only minimal information on non-Apis bees, such as the solitary and stingless bees. These bees are exposed to pyriproxyfen residue on leaf tissues and through soil. Pyriproxifen is absorbed into the soil surface, and soil is a route of exposure to solitary bees that nest underground [106]. Australia has an estimated 2,000 species of native bees, and these include the Leafcutter bees, which cut leaves of different forms to protect their nests and build brood cells, a behaviour that would expose them to contact with residue on the leaves [107]. Another group affected would be the stingless bee some of which build nests out of leaves [108].

Native bees are exposed not only by topical exposure, but also orally by drinking water and eating contaminated pollen. Devillers and Devillers (2020) [106] concluded that it is crucial to identify and quantify the effect of pyriproxyfen on native bees, as they have more contamination exposure than the Apis species. There are notable gaps in the literature regarding the effect of pyriproxyfen and s-methoprene on the reproductive success of different bee species and the adverse effects of their metabolites, which according to several studies can be more severe than those of the parent chemical.

Other non-target insects

As discussed in an earlier section, there is a comprehensive list of non-target insects that are affected by contact with, or ingestion of, Insect Growth Regulators. As synthetic compounds that mimic the action of juvenile hormones, these juvenoids block the metamorphosis of insect larvae to reproductive adults [70]. The juvenile hormones maintain the juvenile character of insects while they molt and are constantly produced. However, when the larvae reach early last-instar larvae or pupa stage the synthesis of the hormone ceases and this in turn causes expression of the gene Krüppel-homolog 1 to drop. As the function of Krüppel-homolog 1gene product is suppression of metamorphosis, the development of the larvae to adults can now proceed. When the larvae are exposed to the Insect Growth Regulator, which has the same effect as the Juvenile hormone, this suppression of metamorphosis continues and the larvae cannot progress to adult stage [70].

Methoprene is lethal to termites. The current hypothesis is that for some termite species, toxicity is due to starvation induced by the elimination of the termite's symbiotic protozoa, inhibiting their ability to digest cellulose from timber [109]. Experiments conducted by Haverty and Howard (1979) investigated exposure to termites via s-methoprene soaked pads and found that the toxin killed four major species of specialty protozoa in the termite gut [109].

Birds

In the program's permit for s-methoprene (permit no PER90213) it is acknowledged that chickens are affected, the permit dictates that chickens should be kept away from treated areas for two days. This stipulation suggests that either the corn grit is expected to be consumed by fire ants or other species within two days, or the pesticide is only active for two days. Recall from a previous section (*Persistence in the environment)* found that if the treated area is shaded, s-methoprene will last more than 8.5 days before breaking down to metabolite compounds. This begs the question what happens to chickens and other birds after two days; or the wild bird species who are exposed from day one of treatment?

Treatment of Leghorn chickens with a single oral dose of methoprene labelled with 14C resulted in residual radioactivity in the tissues and eggs [82]. If it affects chickens, we must consider its effect on other captive and wild bird species, such as pheasant, guinea fowl, waterfowl, quail, partridge, pigeon, corellas and species with declining populations, such as the Black cockatoo and King parrot.

The European Food Safety Authority (EFSA) [63] determined after evaluating the representative uses of pyriproxyfen sprayed as an insecticide on citrus fruit, pome fruit (apple, pears), tomatoes, ornamentals (field use) and tomatoes, ornamentals (greenhouse application), that low acute and long-term risk from dietary exposure to birds was concluded for all the representative uses. Their assessment had no information on repeat-dose toxicity nor toxicological studies on the metabolites of pyriproxyfen 4'-OH-Pyr (and conjugate), 2,5-OH-PY, POPA, POP sulfate conjugate and PYPA. In the first peer review [110], an acceptable daily intake

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(ADI) of 0.1 mg/kg bw per day was set. However, for the renewal, 0.05 mg/kg bw per day was set based on the 78-week mouse study. Based on increased malformations in a developmental rabbit study, the acute acceptable operator exposure level (AAOEL) was agreed to be 0.4 mg/kg bw per day. Both AOEL and AAOEL have been derived with the application of a UF of 100 and a correction for an oral absorption value of 40% [63].

In the studies associated with the EFSA's report, pyriproxyfen was predominantly found in poultry eggs, muscle, and fat (46–94% Total Radioactive Residue (TRR)) following exposure from *representative uses* of the pesticide, i.e. its presence in poultry feed from source products. Extensive degradation occurred in the liver and kidney, where the pesticide was degraded into numerous minor metabolites, and a significant fraction of radioactive residues associated with proteins. In eggs, liver, muscle and fat, the metabolites 2-OH-PY and 4'-OH-PYR (free and sulfate conjugates) were identified in relevant proportions (> 10% TRR) [63].

There is a lack of studies that investigate the effects of pyriproxyfen or s-methoprene on reproductive viability of birds which have ingested the toxins. However, two important studies have been conducted which administered pyriproxyfen to chicken embryos, for the purposes of researching the effects of this toxin on vertebrate brain and heart development. These are discussed in the following section.

Vertebrates

There is clear and concerning evidence in literature demonstrating that pyriproxyfen and s-methoprene present serious risks to vertebrates, particularly during the embryonic stages of development of the young. The receptors of pyriproxyfen are similar to the receptors of retinoic acid, therefore pyriproxyfen will bind to the retinoic acid receptors [111],[112],[113]. While juvenile hormone controls both the metamorphosis and reproduction in insects [114] [115], retinoic acid, the primary derivative of vitamin A, is essential for the normal regulation of a wide range of biological processes including development, differentiation, proliferation and apoptosis in vertebrates. The binding of retinoic acid to the retinoic receptor at appropriate times during development is vital for expression of the correct genes to support organ development and visual function and to transform cell types from the proliferative profile to the maturation profile by inducing differentiation [116]. Retinoic acid is critical for the production of oocytes and sperm in mammals and has a major role in the embryonic development of fins in zebrafish as well as limbs in amphibians, chicks and mice. Studies have found that binding of pyriproxyfen to the retinoic receptor during development interferes with the activity of retinoic acid, which in turn compromises the gene expression cascades resulting in congenital anomalies [117],[118],[119],[120].

Similar to pyriproxyfen, several studies show impairment of vertebrate development caused by s-methoprene. When the Northern Leopard frog was exposed to a range of different concentrations of methoprene between the development stages of *eggs* through to *forelimbs* visible, severe developmental effects were seen at the highest concentration of methoprene [121].

In addition to causing deformation in frogs, it was also established that 5% methoprene (5 g/kg) was toxic to Gray Treefrog (*Hyla versicolor*) tadpoles. Paulov (1976) [122] found toxic and inhibitory effects of methoprene on the development and metamorphosis of toad tadpoles (*Bufo bufo*).

Animal model experiments have demonstrated that both s-methoprene and pyriproxyfen can induce DNA damage in vertebrates. Harmon et al. (1995) [112] voiced concerns that s-methoprene and its derivatives, in particular s-methoprene acid, can affect vertebrate gene transcription, as evidence shows that similarly to pyriproxyfen the compounds can bind to the retinoid X receptor. Since retinoids act as ligands during vertebrate development, this can be expected to have developmental effects on vertebrates. Schoff and Ankley (2004) [123] showed that methoprene blocks the retinol-induced transcription of RAR/RXR regulated reporter genes, while methoxyl-methoprene acid blocks retinaldehyde stimulated transcription.

Luckmann et al (2021) [11] investigated the effects of pyriproxyfen on the brain and nervous system during vertebrate development via a series of experiments using chicken embryos. The results of their study showed that in almost all measurements, the development of the embryos was affected by exposure to pyriproxyfen, even for the embryos exposed to the World Health Organisation (W.H.O.) safe concentration for drinking water of 0.01mg/L. At a concentration of 10 mg/L pyriproxyfen reduced the thickness of the brain's lining (ependymal layer) and the thickness of tissue in the midbrain and outer brain, as well as causing a decrease in brain cell density. The study concluded that DNA damage, and thereby cell apoptosis, oxidative stress and hormone disruption, were caused by the effect of pyriproxyfen on retinoic acid function.

Conte Bernhardt et al (2024) [124] conducted a very similar study to investigate pyriproxyfen's effects on the heart. Their results were remarkably similar to those obtained by Luckmann et al. [11], reduced thickness of cardiac tissue, DNA breakage and a decline in cell proliferation; however, this study observed a decline in apoptosis, indicating cell cycle arrest. The authors recorded a reduction in body and heart mass, body length, head size and other physiological measurements for embryos exposed to pyriproxyfen-again, even in those embryos exposed to the comparably low dose of the W.H.O. safe concentration for drinking water (0.01mg/L).

Pyriproxyfen exhibited low acute toxicity when administered orally, dermally or by inhalation in rats or mice [63]. Pyriproxyfen also caused a reduction in body weight gain as well as damage to the testicular architecture in mice and thus may potentially interfere with spermatogenesis [96].

Kojima et al. (2007) investigated the estrogenic effects of pesticides, among them pyriproxyfen. They used the E-CALUX assay system, which utilizes human ovarian carcinoma cells (BG1) that are transfected with an estrogen-responsive luciferase reporter gene plasmid. The outcome of this testing established that pyriproxyfen had estrogenic activity [125].

A study by da Silva et al (2024) found that chronic exposure to pyriproxyfen from pre-puberty until adulthood may pose a reproductive risk for females according to the juvenile female rat model they used. Using dosages of 0. 1 mg/kg to 1 mg/kg, which led to reduced thyroid mass and increased liver mass indicting a systemic toxic effect, increased ovarian interstitial tissue and decreased the thickness of the endometrial stroma with reduced content of collagen fibers of the uterus. Pyriproxyfen doses were also associated with a 30% reduction in pregnancies and an increase in fetal deaths in the animals exposed to 0. 1 mg/kg [126]. Taken into consideration that the mice used only weighed 35.to 36 g, the animals would have only consumed 0.0035 mg (3.5 µg) or

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0.0036 mg (3.6 µg) per mouse at 0.1 mg/kg and 35 or 36 µg per mouse at the 1 mg/kg.

Truong et al. (2016) [127] stated that pyriproxyfen has a very low solubility, high hydrophobicity and partition coefficients, and is therefore easy to store in fat for the long term. This insinuates a potential toxicity risk for pyriproxyfen in animals. *In vitro* studies performed with rat duodenum strips in the presence of doses of 0.032 – 100 µM (20.3 µg/L- 32.2 mg/L) pyriproxyfen showed that pyriproxyfen affected the activity of duodenum and jejunum smooth muscle strips, with the reaction always being myorelaxant and dose dependent [128]. Rats are a model organism for assessing pesticide induced toxicity [129], which makes the outcome of measuring cell proliferation toxicity to rat heptocytes, apoptosis and DNA damage induced by pyriproxyfen and its metabolites suggesting that metabolites had a higher toxicity than pyriproxyfen in rat hepatocytes [13] particularly alarming.

Although the metabolite formations of pyriproxyfen and s-methoprene appear reasonably well-known, their effects on vertebrate development are little studied, with the focus being mainly on the parent compounds. Available research shows that pyriproxyfen and s-methoprene metabolites can affect vertebrate hormone production, immunity and liver function.

Degitz et al (2003) [130] looked at the developmental effects of the degraded compounds of s-methoprene, such as s-methoprene acid, s-methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid. They discovered that even though exposure to 0.5 mg/L of s-methoprene did not affect the development of *Xenopus laevis* (frog) embryos, all but one of the degraded compounds (7-methoxycitronellic acid) affected development in the range of 1.25 mg/L to 5 mg/L.

As stated previously, 4'-OH-pyriproxyfen, one of the most commonly formed metabolites of pyriproxyfen, aggregates in the liver and adipose tissue. Recent studies have found that 4'-OH-pyriproxyfen is an active antagonist of thyroid hormones [131]. The study by Vancamp et al. (2021) exposed transgenic tadpoles expressing thyroglobulin to 4'-OH-pyriproxyfen for 72 hours and found that even at low concentrations (10-7 nM) there was a decrease in thyroglobin, the precursor of thyroid hormones (T3 and T4). These hormones are vital for neurological and cognitive development [132] and it is no surprise that high doses (10^{-1} mg/L) caused low mobility while at doses of (3×10^{-1} mg/L) decreased head size and disproportionate dimensions of the forebrain and midbrain were observed [131]. The results from these experiments were not available during the European Food Safety Authority's approval process for pyriproxyfen (2019) which took place two years earlier, and the open European Food Safety Authority (EFSA) concluded a low risk from 4'-OH-Py via secondary poisoning to mammals in the context of representative uses on citrus, pome fruits and tomatoes [63].

The EFSA is aware of the uptake of pyriproxyfen metabolites into the bodies of vertebrates. In testing conducted by the EFSA (2019) [63] labelled pyriproxyfen given to ruminants was extensively degraded in milk, liver and kidney (< 1–15% TRR). The predominant degraded product in those matrices was 4'-OH-PYR (free and sulfate conjugates) (18–53% TRR). Hence, the metabolite identified by Vancamp et al. (2021) [131] to adversely affect thyroid hormones necessary for healthy development of young was found in milk. Other main compounds tracked for biodistribution were 2,5-OH-PY (conjugate) in milk (30% TRR), POPA in liver (16% TRR) and POP sulfate conjugate in kidney (36% TRR). In the muscle, the parent compound and 4'-OH-PYR (free and sulfate conjugated) were predominant (44% and 22% TRR, respectively), while in fat, the parent compound was the dominant compound, followed by 4'-OH-PYR (79% TRR and 30% TRR, respectively). From this study, the dietary burden for ruminants was calculated, and the default residue definition for monitoring and risk assessment for animal matrixes for pyriproxyfen was set. The EFSA's conclusion was that the transfer of total residues was insignificant in milk and tissues (< 0.01 mg/kg). They also concluded a low risk from 4'-OH-Py via secondary poisoning to earthworm, fish-eating birds and mammals for all the representative uses on citrus, pome fruits and tomatoes [63].

The Joint FAO/WHO Meeting on Pesticide Residues established an acceptable daily intake of 0–0.1 mg/kg body weight. This data was based on two one-year studies of toxicity in male dogs, taking into account the increased relative liver weight and increased total plasma cholesterol concentration using a safety factor of 100 [16].

The transfer into milk is also apparent in s-methoprene [84]. Studies done decades ago suggest that in mammals radio-labelled methoprene was excreted via faeces and milk in cows, with some radioactivity also measured in tissue [84]. These findings suggests that animals grazing on bait-treated pasture, and thereby absorbing pyriproxyfen and s-methoprene, will break down the parent chemical to its metabolites- the most prominent of which have been shown to interfere with development- storing them in tissue (meat) and excreting them in milk.

There are also indications that Insect Growth Regulators can damage animal and human immune systems. According to Kensler et al. (1978) [133], juvenile hormones inhibit the mitogenesis of bovine lymphocytes, the white blood cells that serve as T cells, B cells and natural killer (NK) cells for immune response. A study by Lamberti et al. (2014) [134] suggests that pyriproxyfen is strongly cytotoxic to human hepatocytes (liver cells). This concurs with the chemical's toxicity label, which lists general human health issues such as possible liver toxicants, possible blood toxicants and endocrine issues, such as an estrogenic effect [135].

Based on the research cited above, the use of pyriproxyfen and s-methoprene, particularly in the broad scope of the fire ant treatment program, poses serious risks to humans and animals, especially for infants. The evidence of several modes of potential damage to the development should invoke concern and trigger review by global government safety bodies- and in Australia the APVMA- regarding the scope of overall use of the pesticides.

Observation and call for precautionary principle

The general population is exposed to methoprene via dermal contact with consumer products containing methoprene (PubChem). Insecticide exposure in animals can occur through mouth, skin, inhalation or maternal routes [20]. Spray drift of a helicopter exposes a person/animal to the growth regulator via inhalation and skin contact. Bait landing in dams, water troughs or puddles exposes animals to the pesticide via the oral route or via the skin.

The risk assessment conducted by the APVMA (Australian Pesticides and Veterinary Medicines Authority) for the NFAEP program is unavailable to the public and could not be included in this literature review. The food safety risk assessment conducted by the European Union detailed many gaps and several areas were

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deemed impossible to assess, despite its conclusion permitting continued use within their described applications. Any consideration of a significant increase in application and/or a novel use of pyriproxyfen and/or s-methoprene should solicit the employment of the precautionary principle. The authors were unable to find, and assume the absence of, any studies conducted to infer the safety of broadscale IGR use over an area of a significant magnitude. With numerous studies indicating negative effects not only on insects, but on soil organisms, amphibians, birds and mammals, the existing literature presents sufficient basis for adherence to the precautionary principle. The precautionary principle means no action should be taken unless we are certain there are no possible negative consequences.

There is a balance in the ecosystem where all animals, insects and even micro-organisms play a role. All animals and plants in the ecosystem co-exist and form a web of great complexity [136]. By poisoning some species, such as ant species and frogs, this balance is disturbed, which may lead to degradation of the ecosystem.

Our agriculture and hence our food production depends on healthy ecosystems for:

- pollination, which is not only performed by bees, but many more insects such as ants, flies, wasps, beetles, moths and butterflies.
- soil health –if soil enzymes of the soil are destroyed, the microbial diversity is reduced and earthworms are poisoned
- pest control – with the ecosystem out of balance there is no control of pest populations

Amphibians, such as frogs, have very permeable skin through which they absorb oxygen as well as toxins. They are an ideal indicator species for environmental pollution, and any observed excess mortality of frogs should be an alarm bell indicating environmental crisis [137].

Summary

In summary, the effects of the NFAEP program, besides most likely failing to achieve the targeted eradication of fire ants as based on the outcome of the last 24 years in Australia and outcomes in the U.S, will be detrimental to the soil, the water, wildlife and the health and stability of the ecological balance. The fact that the pesticides can be stored in adipose tissues indicates an impact on predators, which will most likely lead to a decline in predator numbers much later. A recent review by Cabra; et al. (2024) [62] also concluded that besides the direct toxicity to non-target species, the indirect effects are a shift in food web dynamics and ecosystem functioning. This can potentially destabilize community structure and ecosystem equilibrium by reducing the insect population and disrupting food availability for higher trophic levels [62].

The indiscriminate spraying of pesticides over a large area must be put on hold until proper safety studies are done to determine the effects of the pesticides and their degraded compounds on animals, plants, and the environment. The current literature seems to question the safety of such a large-scale approach, and the precautionary principle should be applied.

In the meantime, the program can switch to a targeted approach through surveying on foot or by helicopter (apparently an option that will be used for five years after two years of spraying) and target only the nests with pesticides or preferably alternatives to pesticides.

There are effective alternatives to pesticide. The recommended biological controls by IFAS Extension University [138] of Florida include preservation of other ant species and introduction of natural enemies of the fire ants to the area. Little black ants have been observed to pull fire ants apart (personal observation). For mechanical control pouring very hot or boiling water on the mount via steam injection or pressure devices is fairly effective. Back in 2007 it was already stated by entomologists that "elimination of fire ants cannot and should not include the use of poison baits as they are not specific to fire ants or even ants", but instead should rely on the use of hot water [139]. The water is heated to 66ºC or higher and applied with pressure to the mount. Associate Professor Joshua King from the University of Central Florida has developed a device and technique that provides large volumes of 100 ºC water to apply to mounts and instantly kills the ants [140] [141]. Professor Nigel Andrews from the Southern Cross University at the Gold Coast in Australia has validated the hot water injection methods in a pilot study [142]. Another method that might be effective according to the IFAS is citrus oil, e.g. orange oil, containing d-limonene which is toxic to fire ants and is the active ingredient of one commercial fire ant killer [138].

Statement and Declarations

Acknowledgements

We like to thank Associate Professor Matt Landos for editing part of the manuscript. Thank you Brett Nagel for providing photo 1.

Funding

No funding was obtained for this study

Competing Interest

There are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication by any of the authors.

Author Contribution

All Authors contributed to writing this review. All authors read and approved the final manuscript.

Ethics approval

No ethics approval was needed

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