Final Report

Environmental Monitoring of Locust Control Operations in Malaimbandy, Madagascar

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Final Report

Environmental Monitoring of Locust Control Operations in Malaimbandy, Madagascar

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Table of Contents

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Chapter 1:

Summary

An environmental impact study of locust control operations was conducted in Malaimbandy, Madagascar, to monitor short and medium-term effects of fipronil (Adonis® 4 UL) and deltamethrin (Decis® 17.5 UL) on terrestrial non-target organisms. The study lasted from the beginning of the dry season in May to the onset of the rainy season in November 2000. Full cover aerial treatments were applied under operational conditions to plots measuring 100 ha each in a replicated block design. The pesticides were sprayed at field doses between 3.2-4 g (fipronil) and 14-15 g a.i./ha (deltamethrin). The number of replicate plots was four, three and two for deltamethrin, control and fipronil, respectively. Terrestrial invertebrates (epigeal and vegetation-dwelling arthropods, flying insects, the mound-building termite *Coarctotermes clepsydra*) and vertebrates (insectivorous lizards and mammals) were monitored over a six month period, starting one week before treatment. Incidental observations on acute toxic effects on aquatic macro-invertebrates were also made.

Both pesticides caused massive kills of freshwater shrimp, but populations seemed to have recovered at the onset of the rainy season. The acute toxicity of deltamethrin to crustaceans is well known, but fipronil was hitherto considered as a product which is not hazardous to aquatic macro-invertebrates at recommended dose rates. The results from this study suggest that the current risk classification of fipronil has to be revised.

The acute toxicity to epigeal and vegetation-dwelling insects was very similar among treatments, with median population reductions of ≈ 70 percent in the fipronil and ≈ 80 percent in the deltamethrin treatment. In contrast, fipronil was more toxic to spiders (≈ 60 percent) than deltamethrin (no significant effect). Moreover, with the exception of springtails, the effects of fipronil were generally more persistent. Deltamethrin did not affect *C. clepsydra*, but fipronil had a strong effect on this harvester termite, killing 80-85 percent of all colonies. No recovery was observed until 24 weeks post-treatment. Side-effects on other termite species were not monitored.

A significant decline of populations of two insectivorous reptiles, *Mabuya elegans* (Scincidae) and *Chalarodon madagascariensis* (Iguanidae), was noted in the fipronil treatment, ranging from 61 percent to 84 percent between 8-24 weeks post-treatment. No significant population reduction occurred in the deltamethrin treatment. Equally dramatic effects were observed in the lesser hedgehog tenrec, *Echinops telfairi* (Tenrecidae), which was not found in plots treated with fipronil. Analysis of the diet of these animals showed that termites, including *C.* clepsydra, are an important prey item, especially for M. elegans and E. telfairi, but also for the large-eared tenrec, Geogale aurita. Regression analysis revealed a significant relationship between the density of viable C. clepsydra colonies and the density of M. elegans and E. telfairi. The results provided evidence of a disruption of the food chain due to fipronil

resulting in an overall depletion of invertebrate prey (termites, epigeal arthropods) and a subsequent decline of several insectivorous vertebrates.

This study confirmed an extraordinary toxicity of fipronil to termites and provided firm evidence of the role of *C. clepsydra* as a keystone taxon which is vital to the functioning and preservation of the food web. Given the magnitude and duration of indirect effects on vertebrate populations, the use of fipronil, an otherwise highly efficient locust control agent, is no longer environmentally acceptable in Madagascar.

Chapter 2:
Recommendations

1. Fipronil should no longer be used as a full cover spray against locusts in Madagascar.

2. Its use as a barrier spray should be suspended unless proof has been given that direct or indirect hazards to the non-target fauna, in particular termites and vertebrates, are transient, i.e. that affected communities recover from the hazards incurred.

3. ONE is encouraged to initiate and seek funding for follow-up studies in areas treated with fipronil during the 1996-1999 migratory locust plague to investigate the state and recovery of affected non-target populations and functional processes mediated by termites. Emphasis should be given to “high impact zones”, i.e. areas subjected to full-cover or replicate barrier sprays.

4. Baseline data should be elaborated on the diet of insectivorous vertebrates in locust areas, and the role of the termite fauna in the food web. These studies aim to improve future risk assessment and management procedures in Madagascar so as to *predict* rather than *trace back* environmental hazards. On the basis of a better understanding of ecological key functions in affected food webs, informed and balanced decisions on the use of pesticides can be taken by Malagasy pesticide registration and environmental protection authorities.

5. The current classification of fipronil as an agent posing a low risk to aquatic invertebrates should be revised. This requires that toxicity tests with shrimp or other decapod crustaceans be conducted. Such tests can be entrusted to the *Centre de Recherches en Écotoxicologie pour le Sahel* (CERES/LOCUSTOX) in Senegal which maintains a certified laboratory for invertebrate toxicity tests.

6. Most anti-locust pesticides threaten natural and/or commercial shrimp stocks. To reduce the risk to shrimp, it is recommended to establish ecotoxicological profiles for all current and future pesticides registered for locust control in Madagascar.

7. A dialogue without prejudice should be opened among stakeholders involved in locust control, including research and development agencies, plant protection authorities, conservation organizations and industry. FAO could take the lead in bringing these groups together to learn from the “Madagascar case” and to develop control strategies which do not cause environmental hazards as experienced in the present study.

8. Many worthwhile recommendations have been made with respect to the management of migratory locust plagues in Madagascar (World Bank, 1998; FAO, 1999; Tingle

and McWilliam, 1999), and there is no need to reiterate them here. Yet there are two important proposals which should be underlined again:

a. Madagascar is in urgent need of environmentally sound locust control strategies, and an effort should be made by plant protection authorities and the donor community to promote and support biological control approaches.

b. The introduction and large-scale use of new control agents – whether chemical or biological – should be accompanied by environmental impact studies financed through an *Environment Fund* provisioned by suppliers of pesticides (companies and donors) as well as national and international development and funding agencies.

Chapter 3:
Introduction

Between 1997 and 2000, more than 33,000 square kilometers of natural landscape in western and southwestern Madagascar were treated with barrier or full cover sprays of synthetic chemical insecticides to control the Malagasy migratory locust, *Locusta migratoria capito* (Orthoptera: Acrididae). In the beginning of the campaign against swarming locusts, which usually involves total cover treatments of settled swarms, the most widely used agent was fipronil (Adonis® 4 UL), a persistent phenylpyrazole insecticide. It was later replaced by deltamethrin (Decis® 17.5 UL), a pyrethroid with a more rapid knock-down effect. However, a concentrated formulation of fipronil (Adonis® 7.5 UL) was used in large-scale barrier treatments against hopper bands from 1998 to 1999, in particular in the migratory locust recession area in southwestern Madagascar.

The environmental impact of locust control operations during the last plague was first studied in Ankazoabo in 1998. The study on non-target effects of fipronil as a barrier treatment (7.5 g a.i./ha within barriers) revealed a severe and long-lasting decline in the vigor and population density of the mound-building termite *Coarctotermes clepsydra*[1] (Isoptera: Termitidae) (ONE, 1999; Tingle and McWilliam, 1998, 1999). A recent evaluation in November 2000 showed that – 33 months after treatment – barriers were not yet fully re-colonized (Zehrer, personal communication). A long-term decline of species of *Microcerotermes* and *Psammotermes* due to fipronil (≥ 10 g a.i./ha) was also reported from Senegal (Danfa et al., 1999). These termite genera – while well represented in Madagascar – have not been monitored in environmental impact studies on the island. No other insect or spider taxon appeared to be as sensitive to fipronil as *C. clepsydra*. However, there were – as yet unconfirmed – indications of hazards to reptiles and other vertebrates (Tingle and McWilliam, 1999).

The first evidence of serious effects on keystone insects such as termites, which are considered vital to the functioning of tropical ecosystems, lead to the call for an immediate withdrawal of fipronil from the Malagasy acridicide market (BBC, 2000; Dinham, 2000; Tingle and McWilliam, 1999). This call, however, was contended by representatives from industry and academia on the argument that the field experiments were not representative. An FAO mission in 1999 conceded that more data are needed before final risk management decisions can be taken and proposed follow-up environmental impact studies which focus on the most controversial issues (FAO, 1999). The mission also proposed that deltamethrin be included in future impact studies, since no data had been gathered in Madagascar so far.

[1] taxonomy according to Cachan (1949, 1951)

In response to these proposals and following a request from the *Office National pour l'Environnement* (ONE), the United States Agency for International Development (USAID) provided the necessary funds for the present environmental impact study (EIS). The EIS was realized in collaboration between ONE, the *Faculté de Sciences*, University of Antananarivo, and the Institute of Environmental Sciences, NLU-Biogeography, University of Basle, Switzerland. The aim was to monitor and evaluate side-effects of full cover sprays of fipronil (4 g a.i./ha) and deltamethrin (15 g a.i./ha) on terrestrial non-target organisms.

The terms of reference for NLU-Biogeography were:

- to establish the general methodological approach together with the team
- to assist in the identification of an appropriate trial site
- to assist in the analysis and interpretation of the field data
- to edit the intermediate and the final report

The field work was conducted from April to November 2000 in Malaimbandy, western Madagascar. The emphasis was on terrestrial arthropods, including *C. clepsydra*, and insectivorous reptiles and mammals. A description of the experimental design and principal monitoring techniques is given in Peveling (2000). More detailed results and figures for individual biota will be presented in the final report of the University of Antananarivo.

The focus in the present report is on the effect of pesticide-induced food shortages on insectivorous vertebrates. The analysis is based on a representative study of the diet of two reptiles, *Mabuya elegans* (Scincidae) and *Chalarodon madagascariensis* (Iguanidae), in relation to the abundance of – or shortage in – potential invertebrate prey. Preliminary results on the diet of two insectivorous mammals, the lesser hedgehog tenrec, *Echinops telfairi*, and the large-eared tenrec, *Geogale aurita,* (Tenrecidae), are also presented. Moreover, an account will be given of the recovery of freshwater shrimp (Atyidae) in temporary ponds where massive kills had been observed in May 2000 following aerial treatment with deltamethrin and fipronil (Peveling, 2000).

Chapter 4:
Study Area and Design

The EIS was carried out near Malaimbandy, about 200 km southwest of Antsirabe. The first experimental site (block 1) was situated 5 km to the north on the road to Miandrivazo, the second (block 2) 10 km and the third (block 3) 40 km to the west on the road to Morondava. Since CNLA *(Comité National de Lutte Antiacridienne)* was about to close down the Miandrivazo base in the beginning of June, and for other reasons explained in Peveling (2000), an intended fourth block (replicate) had to be cancelled.

Each block contained three plots of 100 ha each (one control and two treated plots). The distance between plots within blocks was at least 2 km. All blocks lay within reach of CNLA spray aircraft based in Miandrivazo which was an important criterion for site selection. Swarm control was still under way during the pre-spray monitoring and the treatment period. Even though no swarms were seen during the field work, plentiful faeces on the ground in some plots provided clear evidence of recent visits. Plots within blocks were further divided into three subplots or transects. These were used to monitor invertebrates (epigeal and vegetation-dwelling arthropods, flying insects, termites) and vertebrates (insectivorous lizards and mammals) over a 6 month period, starting 1 week before treatment (Appendix II).

The present study is exceptional in that it was carried out between the beginning of the dry season in May and the early rainy season in November – as opposed to previous EIS which focused on rainy season treatments. Thus it highlights effects of pesticide stress superimposed on and interacting with natural stressors such as drought and food depletion encountered by the fauna which is still active during the dry season.

The objective of the study was to assess environmental effects of full cover sprays of deltamethrin and fipronil against locust swarms. However, owing to the withdrawal of Adonis® 4 UL for swarm control, this formulation was no longer on stock in Madagascar, nor was the more concentrated formulation Adonis® 7.5 UL. Thus only ≈ 220 l of Adonis® 4 UL could be supplied by CNLA. This quantity was sufficient to treat two plots only.

As a consequence, the fipronil treatment in block three was replaced by another deltamethrin treatment. Therefore, block three contained one control and two deltamethrin plots. The final number of treatments over all blocks was four, three and two for deltamethrin, control and fipronil, respectively, resulting in an unbalanced design. This along with the reduction from four to three blocks certainly reduced the analytical (and statistical) power of the final design, however, the number of replicates was still higher than in most previous environmental monitoring studies of operational scale locust treatments.

Chapter 5:

Treatment

The treatments were carried out under operational conditions on May 11, 22 and 31 in blocks 1, 2 and 3, respectively, using different spray aircraft in each block (two fixed-wing and one helicopter), depending on the availability during the ongoing campaign. Pesticide effects and side-effects may vary in relation to the spray aircraft and atomizers used. The highest efficacy is usually achieved when using computerized track guidance systems (Scherer, 1997, 1998). In Malaimbandy, only one aircraft (block 2) was equipped with differential GPS (see Appendix IV for application details). Nonetheless, judging on the droplet deposition, the overall spray quality was very similar among blocks and spray aircraft (Table 1).

Table 1. Droplet deposition in the treatment plots. Methods are described in Peveling (2000).

	Median # of droplets / cm^2 (min-max; percentage of papers with ≥ 1droplet)			
Orientation of oil-sensitive papers	**horizontal (ground level)**		**vertical (1 m above ground level)**	
	Deltamethrin	**Fipronil**	**Deltamethrin**	**Fipronil**
Block 1	2 (0-11; 67 %)	2 (0-7; 80%)	13 (0-68; 98%)	4 (0-49; 80%)
Block 2	3 (0-16; 91%)	4 (0-14; 98%)	6 (1-80; 100%)	7.5 (1-32; 100%)
[1] Block 3 D A	2 (0-6; 89%)		6 (1-20; 100%)	
[1] Block 3 D B	3 (0-10; 98%)		6 (2-25; 100%)	

[1] both plots in block 3 were treated with deltamethrin (D A and D B)

However, there were some differences with respect to the actual field dosages. The nominal dosages (15 g and 4 g a.i./ha respectively for deltamethrin[2] and fipronil) were only applied in block 2 (see Appendix IV). In the remaining blocks, volume application rates were uniformly set to 0.8 l/ha according to standard practices of the spray companies involved, resulting in field dosages of 14 g and 3.2 g a.i./ha, respectively. Thus three deltamethrin plots were sprayed at 14 g (blocks 1 and 3) and one plot at 15 g a.i./ha (block 2). The final dosages for the two fipronil plots were 3.2 g (block 1) and 4 g a.i./ha (block 2).

The total surface treated was about 100 ha in all but one plot (deltamethrin, block 2). Here the spray runs were extended northward (for unknown reasons), resulting in a sprayed area of 151 ha. Nevertheless, variations in field dose and plot size as experienced in Malaimbandy are unlikely to have altered the principal outcome of the present study.

[2] The registered field dose against swarms of migratory locust is 17.5 g a.i./ha. During the last campaign, it was reduced by CNLA to 14-15 g a.i./ha which gave sufficient control.

Chapter 6:
Impact on Terrestrial Organisms

6.1 Epigeal and vegetation-dwelling spiders and insects

More than 33,000 arthropods were sampled with pitfall traps (epigeal or ground-dwelling arthropods), and about 21,000 with sweep nets (vegetation-dwelling arthropods)[3]. Ants (Formicidae) made up the bulk of the catches in pitfall traps (49 percent), followed by springtails (Collembola; 33 percent), the darkling beetles *Zophosis* spp. (mainly *Z.madagascariensis*; Coleoptera: Tenebrionidae; 14 percent) and spiders (Araneae; 3 percent). The vegetation-dwelling fauna was dominated by spiders (31 percent) and plant or leaf hoppers (Homoptera; 29 percent). Bugs (Heteroptera) and crickets and grasshoppers (Orthoptera) represented 12 percent each of the total catch. Within the orthopterans, crickets (Gryllidae; 52 percent) were more abundant than grasshoppers (Acrididae; 26 percent).

The effects of deltamethrin and fipronil on these non-target arthropods are summarized in Tables 2 and 3 (see Appendix III for a description of statistical methods).

[3] These totals comprise the most abundant taxa only.

Table 2. Mean effect of deltamethrin on vegetation-dwelling (no mark) and ground-dwelling (g) spiders and insects; significant effects are printed in bold; effects ≤ 0 percent omitted for clarity.

Taxon	Effect (%)									
	1st week		8th week		16th week		22nd week		24th week	
Araneae										
All spiders	32		40		11		**31**	*		
All spiders (g)	1						21			
Araneidae										
Oxyopidae	53		**66**	*	27					
Salticidae	38	#	20		31				30	#
Salticidae (g)	38				52		57			
Insecta										
Collembola (g)	**22**	*			**50**	**	**91**	***	**88**	***
Orthoptera	**78**	**	48		30		**29**	*		
Acrididae	**64**	*	45		57	#	**47**	***	**31**	*
Gryllidae										
Homoptera	**73**	***	37				**20**	*	**3**	*
Heteroptera	**63**	***			7		8			
Coleoptera	**86**	***	25		33					
***Zophosis* spp. (g)**	**81**	**	1							
Caterpillars	**90**	***	60		**96**	***	**93**	***	**68**	**
Brachycera	**91**	**								
Hymenoptera:										
Formicidae	**86**	**	6						40	
Formicidae (g)										
Other hymenopterans	86								41	

= $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Deltamethrin did not have notable effects on spiders. Significant reductions were only observed on two sampling dates in lynx spiders (Oxyopidae; 8 weeks post-treatment) and Araneae (22 weeks post-treatment). Our results confirm the known fact that spiders are generally less susceptible to pyrethroid insecticides than insects. The acute toxicity to insects was much more pronounced and ranged from 22 percent in springtails (Collembola) to 91 percent in flies (Brachycera including Tabanomorpha, Asilomorpha and Muscomorpha). In hymenopterans, the acute effect was as high as 86 percent, but this value was not statistically significant. Most insects recovered quickly from the initial population decline and no significant reduction was noted 8 weeks post-treatment. However, springtails and caterpillars as well as – to a lesser degree – grasshoppers and homopterans were further reduced during the remaining sampling period. The delayed reduction of vegetation-dwelling insects appeared to be at least partly due to the occurrence of bush fires (Chapter 6.5).

Table 3. Mean effect of fipronil on vegetation-dwelling (no mark) and ground-dwelling (g) spiders and insects; significant effects are printed in bold; effects ≤ 0 percent omitted for clarity.

Taxon	Effect (%)									
	1st week		8th week		16th week		22nd week		24th week	
Araneae										
All spiders	**60**	**	39	#	**78**	**	**49**	**	50	#
All spiders (g)	68		53				**74**	*	69	
Araneidae							25			
Oxyopidae	**78**	**	**94**	***	**95**	***	**73**	**	**99**	*
Salticidae	**81**	***	**63**	*	**82**	***	**46**	*	**74**	*
Salticidae (g)	74		**88**	*	**92**	*	85	#	61	
Insecta										
Collembola (g)	35		24		43		**87**	**	74	
Orthoptera	**71**	***	**89**	***	**91**	***	**91**	***	**76**	**
Acrididae	**48**	***	**100**	***	**95**	***	**90**	***	**78**	***
Gryllidae	79						**79**	**	63	
Homoptera	**86**	***	38		20		10		50	
Heteroptera	**65**	*					19			
Coleoptera	**69**	*	55		33		**83**	**		
***Zophosis* spp. (g)**	**63**	**	**60**	**	**62**	**			**19**	
Caterpillars					**63**	*	**76**	**	66	#
Brachycera	25						11		1	
Hymenoptera:										
Formicidae	**92**	**	**89**	*					17	
Formicidae (g)			34						19	
Other hymenopterans	50				68				14	

= $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Contrary to deltamethrin, fipronil caused a long-lasting population decline in spiders (Araneae). Lynx spiders and jumping spiders (both ground and vegetation-dwellers) were particularly affected and did not recover until the end of the trial (Figure 1). Orb-web spiders (Araneidae) – on the other hand – were not affected. The initial impact on insects was comparable to that of deltamethrin, with significant effects ranging from 65 percent (Heteroptera) to 92 percent (ants). However, as in spiders, the effects were more persistent in crickets and grasshoppers (up to 24 weeks post-treatment) and *Zophosis* spp. (up to 16 weeks post-treatment; Figure 2). Ants were significantly suppressed for 8 weeks (sweep-net catches). This effect was not confirmed for epigeal ants sampled with pitfall traps. However, pitfall traps are known to yield strongly biased results in case of social insects such as ants.

In conclusion, the suppression of – mainly detrivorous and herbivorous – arthropods was more pronounced in the fipronil than in the deltamethrin treatment. This reflects the higher persistence of fipronil. Among the taxa reduced for a longer period of time were at least four which play an important role as natural prey of insectivorous vertebrates: spiders, ants, *Zophosis* spp. and crickets (see 6.4).

Web-building wolf spiders (Lycosidae) dwelling along water courses and small ponds were rarely encountered during the last sampling period in November 2000. Therefore, possible recovery from the severe reduction due to deltamethrin and fipronil observed in May 2000 (Peveling, 2000) can only be assessed later in the rainy season.

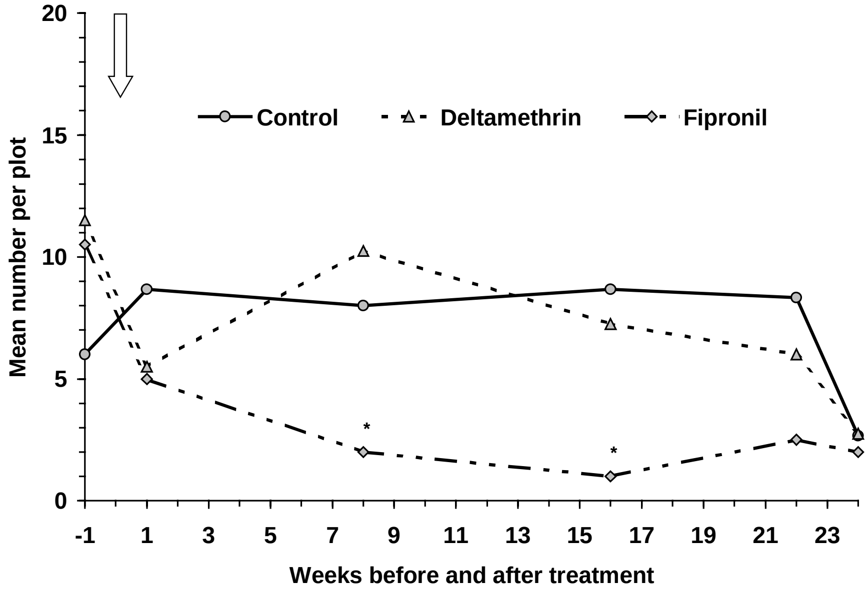

Figure 1 Mean relative abundance of epigeal jumping spiders (Araneae: Salticidae). Significant differences between fipronil and control are marked with * ($P < 0.05$). The suppression due to deltamethrin was not statistically significant. The arrow denotes the time of treatment. [a]

[a] Only two diagrams (Figure 1 & 2) are shown as examples.

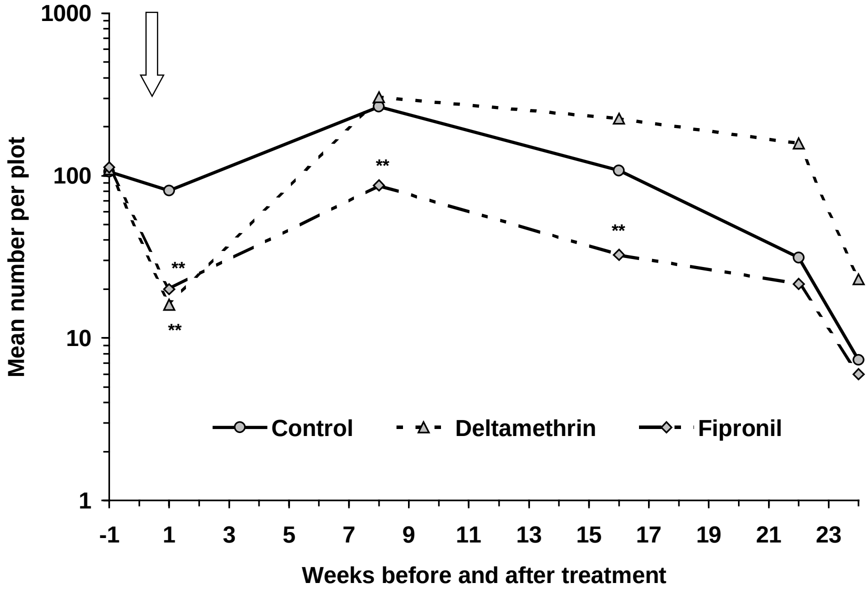

Figure 2 Mean relative abundance of epigeal *Zophosis* spp. (Coleoptera: Tenebrionidae). Significant differences between treatments and control are marked with ** ($P < 0.01$). The suppression due to deltamethrin was only significant during the 1st week post-treatment. The arrow denotes the time of treatment.

6.2 Coarctotermes clepsydra

Different methods to assess the impact of pesticides on termite activity have been practiced in Madagascar since 1995. Andrianaivo (1995) dug holes measuring 7 x 7 cm into mounds of *C. clepsydra* and measured the *time to repair*, i.e. the complete “re-plastering” of the hole. Tingle and McWilliam (1998) and ONE (1999) estimated the *degree of repair* of a hole 10 to 15 cm across and 20 cm deep marked with a flag pole, using a five-score classification system (from 0 = no repair to 4 = full repair, i.e. flag pole completely surrounded by earth). The latter method was also used in Malaimbandy. Field inspection showed, however, that the type and degree of repair – and the concomitant scoring of termite activity – can be quite variable depending on the diameter of the flag pole, the position of the hole and the time elapsed between the damage and the evaluation. Functionally, full repair is achieved if a colony manages to seal all openings so as to maintain the temperature and humidity regimen in the nest. In a healthy colony, this is done during the night after the damage has been inflicted – or even at daytime on cloudy days. Whether or not a large hole is closed to the rim or only sealed on the interior surface is of minor importance. Our analyses showed that only about 30-40 percent of healthy colonies which managed to coat the interior surface within a day conducted a complete repair after 3 days.

Therefore, we translated the 5-score system into a more robust binary system (classes 0-3 = class I, classes 4 and 5 = II). Class II corresponds to a repair > 50 percent according to the old system. We then calculated the percentage of *C. clepsydra* colonies which managed to conduct a "class II repair" (= healthy colonies). Our results confirm the outstanding toxicity of fipronil to *C. clepsydra* even at a very low dose (Figure 3). The percentage of healthy colonies was as high as in the control during the first week post-treatment but dropped significantly to levels < 10 percent after 8 weeks. No recovery was observed until November 2000, i.e. 24 weeks post-treatment. In the other treatments, the rates fluctuated between 95-100 percent (control) and 88-97 percent (deltamethrin). Differences between control and deltamethrin colonies were not significant at any time.

To verify these findings, another 20 *C. clepsydra* termitaria per plot were randomly selected and damaged in a standardized manner. A hole measuring 10 cm in diameter and 20 cm deep was dug into the mound with a machete on the side facing South (Photos 1-2, Appendix I). The position of the hole was halfway between the tip of the mound and the ground. The colonies were visited again one and three days later and the kind of repair – interior surface coated or hole fully sealed (Photo 3) – was monitored. Mounds not showing any repair were dug up to find survivors of *C. clepsydra* or invertebrate invaders.

The analysis confirmed that failure to repair damaged mounds usually indicates colony mortality (Figure 4). In some mounds, however, surviving *C. clepsydra* were found deeper in the nest. Here the population size was obviously reduced to such an extent that repairs could no longer be conducted. Other clear indications of extinct or strongly reduced populations were small holes and channels perforating the outer layer of the termite mound (Photos 2 and 4). Deserted mounds were often occupied by ants, silverfish (Thysanura) or millipedes. Moreover, *Gibbotermes mandibularis*, a termite which seems to live in close association with *C. clepsydra*, was found in 35 percent of the mounds deserted by *C. clepsydra*. It is possible that this species was either less sensitive or – more likely – less exposed to fipronil.

The main route of uptake of fipronil is through feeding on vegetable matter. Therefore, species feeding on wood or dung are expected to collect a smaller – if any – amount than those specialized on grass and litter, since most of the pesticide deposits on grass. This might explain why the humivorous *G.mandibularis* was still alive when colonies of the grass-feeding termite *C. clepsydra* were killed. An aggravating factor is the photo-transformation of fipronil into desulfinyl fipronil, a derivative which is more toxic and persistent than the parent compound (Hainzl et al., 1998; PAN, 2000). The rapid formation of desulfinyl fipronil and concomitant increase of toxic load of the grass cover was certainly favored by the prevailing climatic conditions at the beginning of the dry season (bright sky; high UV radiation).

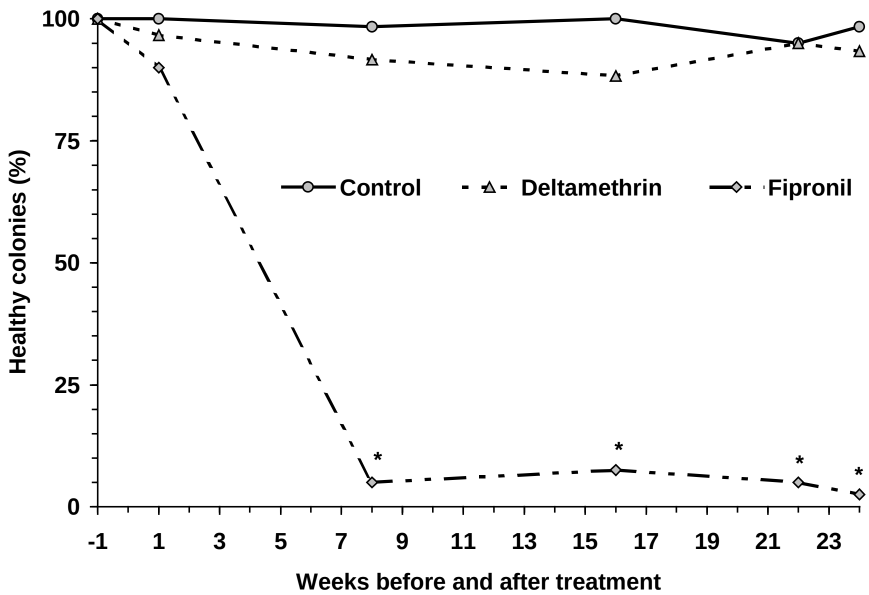

Figure 3 The effect of deltamethrin and fipronil on *Coarctotermes clepsydra*. The diagram shows the mean percentage of healthy colonies from May to November 2000; significant differences among control and fipronil marked with * (P < 0.05); differences between deltamethrin and control not significant.

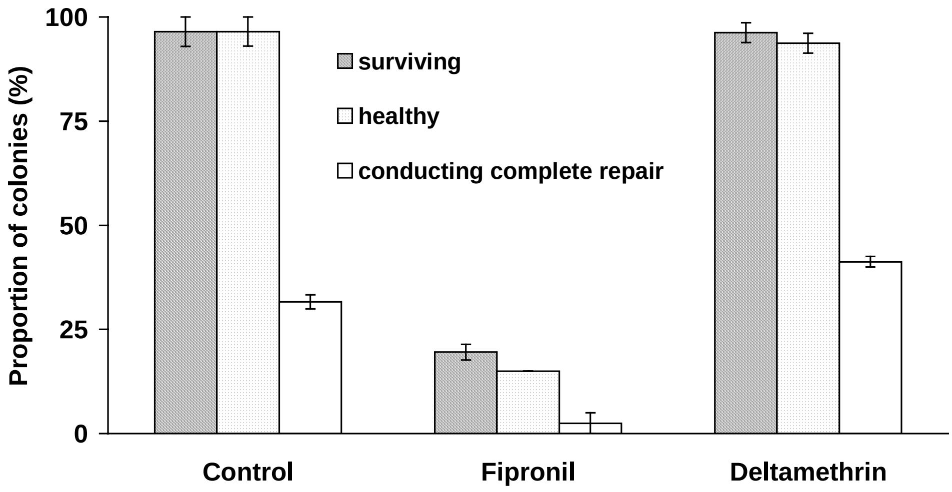

Figure 4 The effect of deltamethrin and fipronil on *C. clepsydra*. Columns are the percentage of surviving and healthy colonies and colonies conducting complete repair of holes within 3 days. Colony survival in the fipronil treatment was 20 percent. Differences between fipronil and control/deltamethrin are significant respectively at $P < 0.01$ (survival, health) and $P < 0.001$ (complete repair); bars are ± 1 standard error.

The first results from residue analysis of vegetation samples from a plot treated with fipronil (block 2) revealed an initial environmental concentration between 5 µg a.i./kg (*Heteropogon contortus*, dominant grass species) and 27 µg a.i./kg (leaves of *Terminalia mantali*, dominant tree species; results to be confirmed in subsequent analyses)[4]. Six months after treatment, the concentration of fipronil in plant matter collected from ten mounds of *C. clepsydra* colonies killed by fipronil was 0,1-0,3 µg/kg (PTRL, 2000)[5], i.e. between 0.4 percent and 6 percent of the initial concentration on vegetation outside of the mounds. This indicates that the break-down of fipronil within mounds, i.e. in an environment with strong temperature and humidity fluctuations (Pearce, 1997), but protected against ultraviolet radiation, was relatively low. However, concentrations of the principal metabolites of fipronil (MB046136, MB045950, MB046513, RPA200766) were below detection limits.

[4] *Centre National de Recherches sur l'Environnement, Département Environnement et Qualité de Vie*, Antananarivo;

[5] Laboratory PTRL Europe, Ulm, Germany; residue analysis funded by the German Development Cooperation (GTZ).

6.3 Reptiles and lesser hedgehog tenrec

The average pre-spray abundance of *C. madagascariensis* (Photo 5) and *M. elegans* (Photo 6) was highest in the two fipronil plots (47 [2] and 60 [7] sightings per 3 km, respectively; standard error in brackets) and about the same in control (14 [3] and 39 [6]) and deltamethrin plots (19 [3] and 31 [3]). Sightings rapidly declined in all treatments as the dry season progressed, but the overall decline relative to pre-spray levels was more pronounced in the fipronil treatment. Even in absolute numbers sightings were lowest in the fipronil treatment from 8 (*M. elegans*) and 16 weeks (*C. madagascariensis*) post-treatment. The mean effect values of deltamethrin and fipronil are presented in Table 4, and Figures 5 and 6 show the change in relative abundance compared to pre-spray levels.

Table 4 Mean effect of deltamethrin and fipronil on *Chalarodon madagascariensis* and *Mabuya elegans*. Significant effects are printed in bold. Effects ≤ 0 percent indicate an increase in abundance relative to control and pre-spray levels.

Taxon	Effect (%)									
	1st week		8th week		16th week		22nd week		24th week	
Deltamethrin										
C. madagascariensis	-18	n.s.	-107	n.s.	18	n.s.	42	n.s.	-46	n.s.
Mabuya elegans	-25	n.s.	-24	n.s.	29	n.s.	-50	n.s.	-7	n.s.
Fipronil										
C. madagascariensis	-11	n.s.	75	**	74	***	84	***	71	***
Mabuya elegans	-8	n.s.	61	**	75	***	61	*	76	***

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; n.s. = not significant

From these presentations it is clear that fipronil caused a dramatic and long-lasting decline in the density of the two reptile species. Whereas densities were nearly back to pre-spray levels in both control and deltamethrin plots at the onset of the rainy season, they remained low in the fipronil treatment (Figures 5 and 6). The suppression of reptiles can be due to direct toxicity or food shortages. Unfortunately, analyses of pesticide residues in reptiles are still pending. Thus the entry point of fipronil and its metabolites into the vertebrate food chain cannot be traced back for the time being. However, given the low field dose of fipronil applied in the present study, it is much more likely that *M. elegans* and *C. madagascariensis* suffered from food deprivation due to the suppression of important invertebrate prey (see 4.1).

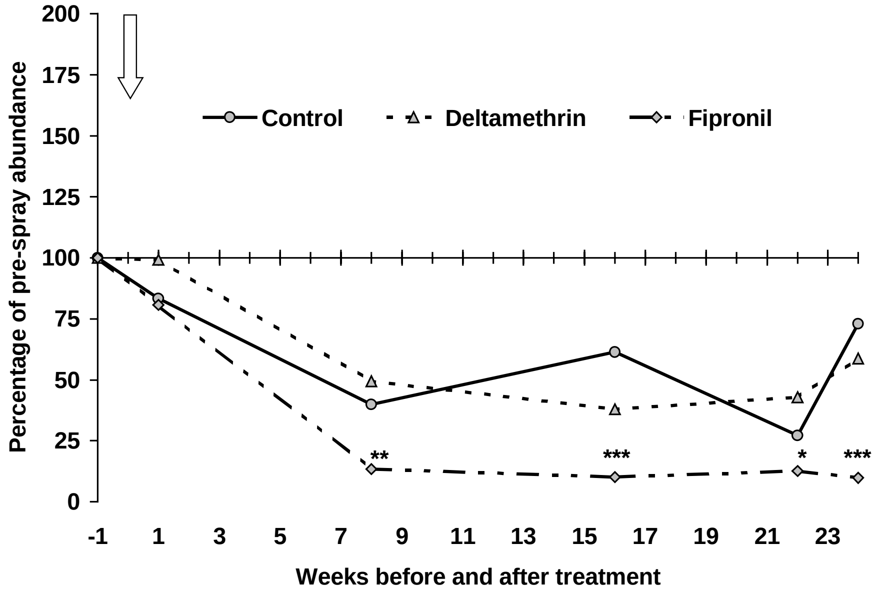

Figure 5 Change in relative abundance of *Mabuya elegans*. Values greater or less than 100 percent indicate an increase or decline in relative abundance compared to pre-spray levels. Significant differences between fipronil and control are marked with asterisks (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Differences between deltamethrin and control are not significant. The arrow denotes the time of treatment.

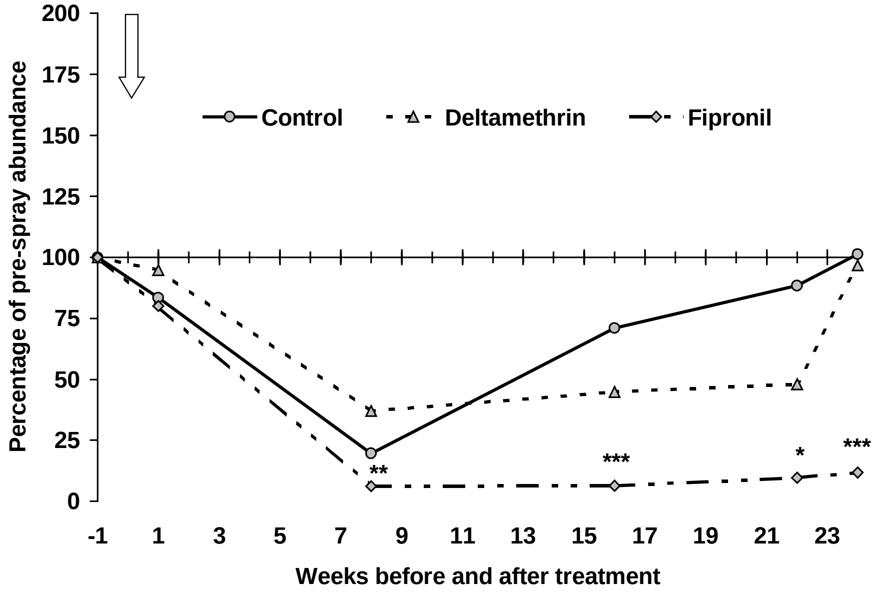

Figure 6 Change in relative abundance of *Chalarodon madagascariensis*. Explanations as in Figure 5.

The overall number of insectivorous mammals caught in Sherman and pitfall traps was relatively low. This was due to hibernation or reduced activity during the dry winter season. For example, the hibernating lesser hedgehog tenrec, *Echinops telfairi* (Photo 7), was not found between May and August but first appeared in September. In this paragraph, we will focus on this species. For the results on other mammals (rodents and shrews), the reader is referred to the final report of the University of Antananarivo. Figure 7 shows the mean total catch of *E. telfairi* in the different treatment groups. Two conclusions can be drawn from the graph. First, *E. telfairi* was not directly exposed to the pesticide treatments. Second, *E. telfairi* was not found in plots treated with fipronil between September and November 2000.

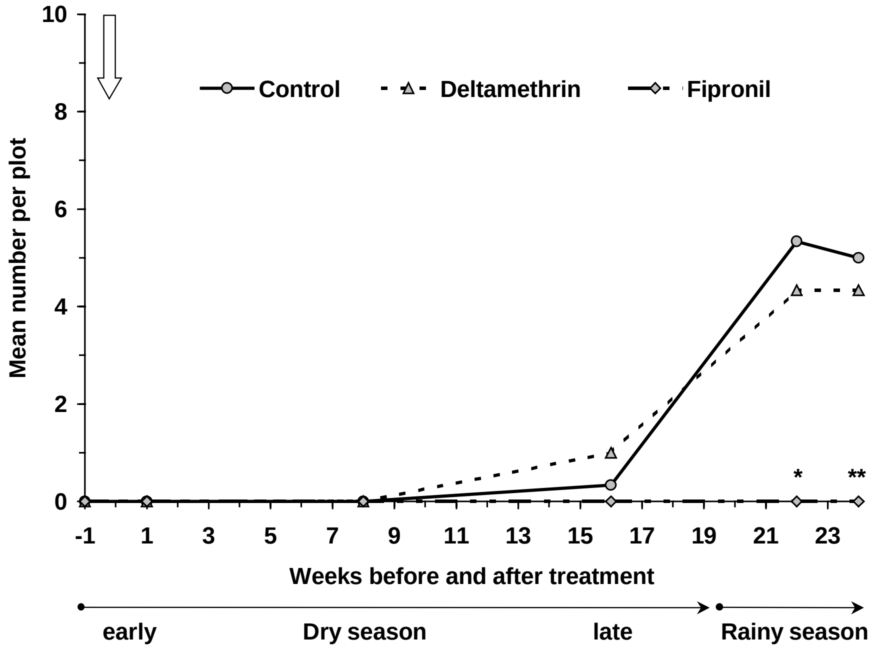

Figure 7. Relative abundance of *Echinops telfairi*. No individuals were found in the plots treated with fipronil. Significant differences between fipronil and control are marked with asterisks (* = $P < 0.05$; ** = $P < 0.01$; one-way ANOVA). Differences between deltamethrin and control are not significant. The arrow denotes the time of treatment.

Our findings suggest that, since direct toxicity of fipronil can be ruled out, the resident lesser hedgehog tenrec population emerging from their hibernation places in the fipronil plots had largely emigrated from the area because of food shortages.

6.4 Invertebrate-vertebrate interaction

6.4.1 Diet of insectivorous reptiles and tenrecs

The long-lasting suppression of epigeal and vegetation-dwelling arthropods and termites due to fipronil clearly suggests a causative relationship between the depletion of the food stock and the decline of insectivorous vertebrates.

To validate this hypothesis, we conducted an analysis of the – as yet unknown – diet of *M. elegans* and *C. madagascariensis*. Between November 3 and 16, about ten individuals of each species were captured in each of the nine plots (between 0900 and 1200 h), yielding a total catch of 90 and 89 specimens of *M. elegans* and *C. madagascariensis*, respectively. Food items were extracted from the stomachs and enumerated on different taxonomic levels. Termites were identified to species and the remaining taxa to order or family (ants). Quantification was achieved by counting the most chitinized body parts (head capsule in insects and cephalothorax in spiders). In a few cases, wings instead of heads were counted. The amount of each prey item was expressed as a percentage of the total number of invertebrates. This mode of quantification overestimates the role of smaller prey such as ants and springtails, whereas less abundant but larger prey such as winged termites or grasshoppers may be underestimated. However, volumetric assessments of the total volume per prey category, as proposed by Cissé and Karns (1977), were not applicable in our study where partly digested body fragments rather than whole bodies were found in the stomachs.

It has to be pointed out that the food preferences reported here reflect the late dry season situation only. In arid and semi-arid environments, there is great inter-seasonal variation both in invertebrate prey abundance and prey preferences of insectivorous reptiles (Cissé and Karns, 1977). Thus, the rainy season diet of the reptiles investigated in our study may be completely different.

In addition to reptiles, we also analyzed the stomach content of two specimens each of *E. telfairi* and *Geogale aurita*. Even though the sample size was certainly too small to draw general conclusions on the food preferences of these tenrecs, we expected to compare our "anecdotal" results with literature reports that termites are among the preferred prey of tenrecs (e.g., Glaw and Vences, 1994).

Our analysis revealed that *M. elegans* and *C. madagascariensis* largely rely on the same invertebrate prey (Figure 8), but differ significantly with respect to the frequency of preferred prey (= frequency of stomach contents dominated (maximum proportion) by a single taxon). This seems to reflect different micro-habitat preferences and resource utilization strategies. The most frequent prey of *C. madagascariensis* were ants (77 percent) and beetles (12 percent)[6]. These are usually hunted on open ground between grass tussocks. *Zophosis* spp. and an unidentified snout beetle (Curculionidae) were the preferred beetle taxa. Termites made up about 7 percent of the diet only, but were the preferred prey of *M. elegans* (57 percent). In this species, crickets (10 percent) and spiders (9 percent) were nearly as frequent as ants (10 percent). *M. elegans* searches its prey in dense vegetation and litter rather than on open ground which explains why crickets, spiders and termites were more frequently caught.

[6] Plant leaves were found in 35 percent of all stomachs of *C. madagascariensis,* and flowers in 17 percent

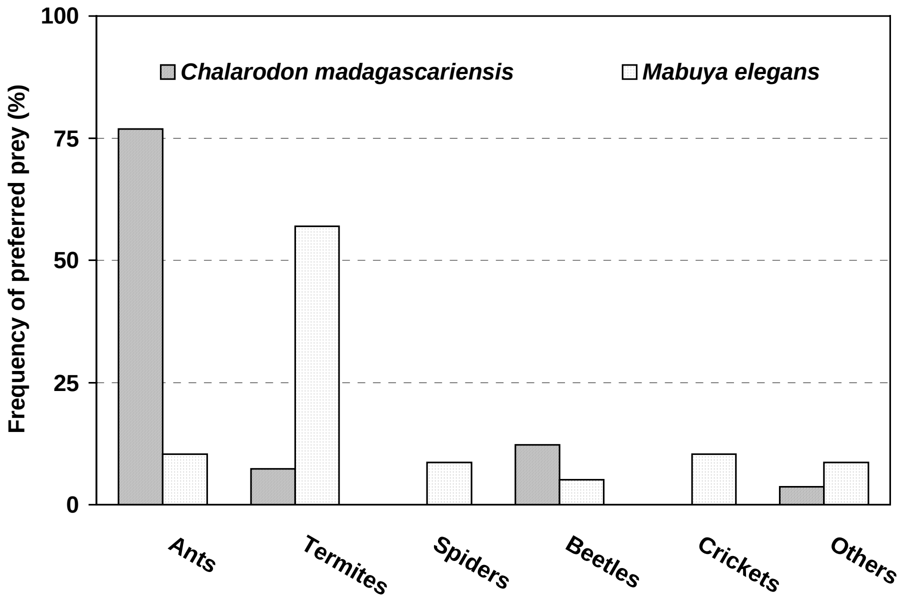

Figure 8 Late dry season / early rainy season diet of *Chalarodon madagascariensis* and *Mabuya elegans* ($n_1 = 82$, $n_2 = 58$). Data are frequencies of stomach contents dominated by a single invertebrate taxon (comprising the highest proportion of all invertebrate prey). Frequency distributions are significantly different at $P < 0.001$ (G-test).

The termite diet was composed of three species: *C. clepsydra* (≈50 percent winged reproductive and 50 percent workers and soldiers), *Gibbotermes mandibularis* (winged reproductives only) and c.f. *Microtermes sakalava* (workers and soldiers only)[7]. The frequencies of these species were approximately the same in *C. madagascariensis* and *M. elegans* (Figure 9). Workers or soldiers of *C. clepsydra* occurred in 2.6 percent of the stomachs from the fipronil treatment, compared to 11.7 percent in the control and 10 percent in the deltamethrin treatment. Low numbers of *C. clepsydra* were no surprise, given the dramatic population decline observed in the fipronil plots. However, heavy rain during the last sampling period led to the immigration of swarming *C. clepsydra* and *G mandibularis* into the plots, and winged termites were found in the stomachs of reptiles from all treatment groups. *M. sakalava* was the most numerous termite species. However, in terms of biomass, *C. clepsydra* workers and winged termites might have been more important food items due their larger body size. Apart from the absence of *C. clepsydra* workers or soldiers in the fipronil treatment, we did not find significant differences in the composition of the diet among treatments. In other words, six months post-treatment, lizards largely relied on the same food resources in all treatments.

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[7] Preliminary identification to be confirmed by termite taxonomists

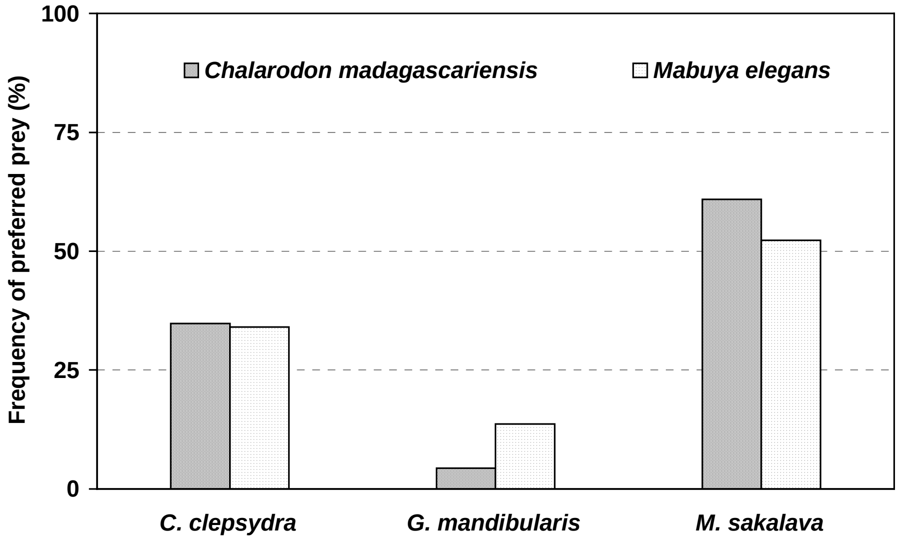

Figure 9 Frequencies of different termite species in the diet of *Chalarodon madagascariensis* and *Mabuya elegans* (n_1 = 23, n_2 = 44). Samples from fipronil plots not included due to the absence of *C. clepsydra* workers and soldiers. Frequency distributions are not significantly different (G-test).

However, this does neither imply that the diet was the same over the whole post-treatment period, nor that the availability of invertebrate prey was similar among treatments. The evidence of long-term reductions of potential arthropod prey rather suggests that food shortages eventually led to a decrease in the carrying capacity for insectivorous reptiles (see Appendix V).

Our observations on the food preferences of tenrecs confirm the particular importance of termites (workers and soldiers) as the principal food (Figure 10). The dominant prey of both *E. telfairi* and *G. aurita* were workers and soldiers of *C. clepsydra*. These regularly leave their nests in large bands during the night to harvest herbs and litter (Andrianaivo, 1995) and make up an easy prey to nocturnal insectivores such as tenrecs.

Surprisingly, we also found remnants of *M. elegans* in both hedgehog tenrec specimens (and another specimen not included in this analysis). While it is possible that skinks were only fed upon when captured in the same traps as tenrecs, we cannot rule out that they are a natural prey. This is supported by reports that *E. telfairi* feeds also on small vertebrates (Anderson, 1986). As a consequence, reduced *M. elegans* densities would be expected to aggravate already existing food shortages resulting from the extinction of more than 80 percent of the *C. clepsydra* population.

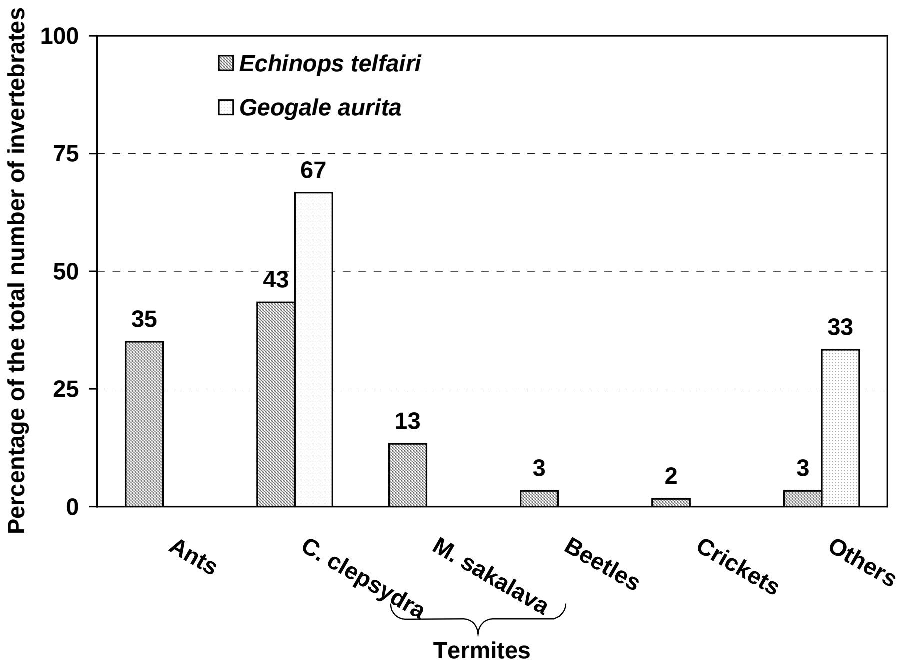

Figure 10 Late dry season / early rainy season diet of *Echinops telfairi* and *Geogale aurita*. Data are the means for two replicate samples ($n_1 = n_2 = 2$).

6.4.2 Relationship between *C. clepsydra* colony density and vertebrate abundance

Termites are regarded as keystone organisms whose well-being is crucial to the functioning of tropical ecosystem (Deshmuk, 1986; Holtmeier, 1999; Tingle and McWilliam, 1999). Our results strongly support this view and confirm devastating effects of fipronil on the most conspicuous termite species, the mound-building *C. clepsydra*. The extent to which other species were affected is unknown, but results from Senegal suggest that termites with more cryptic nesting habits may have been seriously affected as well (Danfa et al, 1999).

The biomass of termites in semi-arid African savannas can be as high as 500 kg/ha (Deshmuk, 1986). Thus, when food is a limiting factor, it can be expected that the density of termitivorous vertebrates depends on the actual termite biomass. For mound-building termites such as *C. clepsydra*, the relative biomass can be inferred from the actual density of viable colonies. In the present study, this was achieved by counting the number of mounds within four randomly chosen circular sub-plots (diameter = 30m) in each plot. Colony survival was investigated as outlined in 6.2. The assessment was conducted during the last sampling period in November 2000 and revealed great differences in viable colony density among treatments. The highest density was observed in the control (41 [8] colonies/ha; standard error in brackets), followed by deltamethrin (27 [9]) and fipronil (6 [1]). Total colony density (viable *plus* extinct colonies) was as high as 41 [5] in the fipronil treatment which means that colony mortality was 85 percent (about the same rate as shown in Figure 4, 6.2). In contrast, total versus viable colony densities were similar both in the control and the deltamethrin treatment.

To investigate the relationship between the density of viable *C. clepsydra* colonies and the density of reptiles and lesser hedgehog tenrecs, we conducted a regression analysis, using a logarithmic model. The analysis did not reveal a significant relationship between viable colony density and the abundance of *C. madagascariensis*. However, the relationship was significant ($P < 0.05$) for *M. elegans* (Figure 11) and very highly significant ($P < 0.001$) for *E. telfairi* (Figure 12). The regression formulas are:

y = 5.2 ln x – 1.4 *(M. elegans)*

y = 6.1 ln x – 11.6 *(E. telfairi)*

where x = the number of viable termite colonies/ha and y = the mean number of sightings 22-24 weeks post-spray *(M. elegans)* or the total catch 16-24 weeks post spray *(E. telfairi)*.

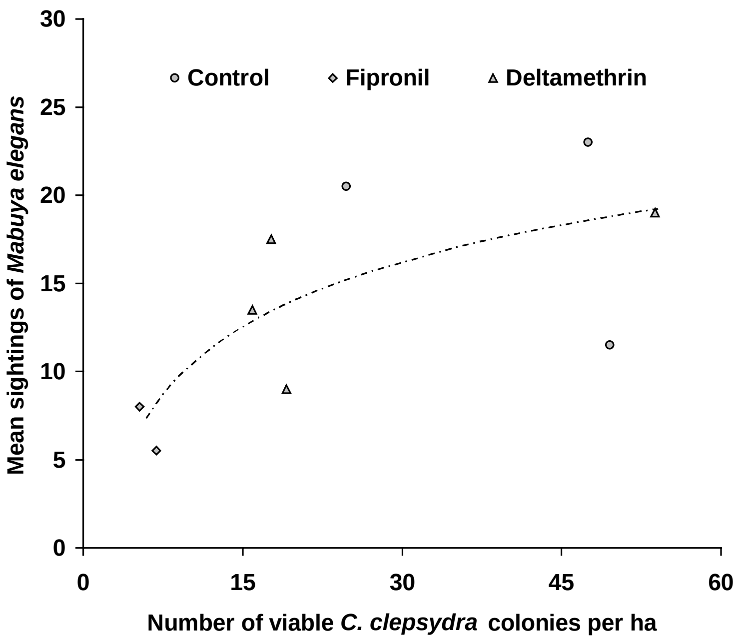

Figure 11 Relationship between viable *Coarctotermes clepsydra* colony density and the relative abundance of *Mabuya elegans*. Data are means for weeks 22 and 24 post-spray.

The regression lines clearly show that low densities of *M. elegans* and *E. telfairi* can be related to low densities of viable *C. clepsydra* colonies. It is noteworthy that, in the case of *E. telfairi*, the regression would also be significant if the fipronil data were omitted. This is a very strong proof of the dependence of *E. telfairi* on *C. clepsydra* as a principal food source and underlines the role of this termite as a keystone species.

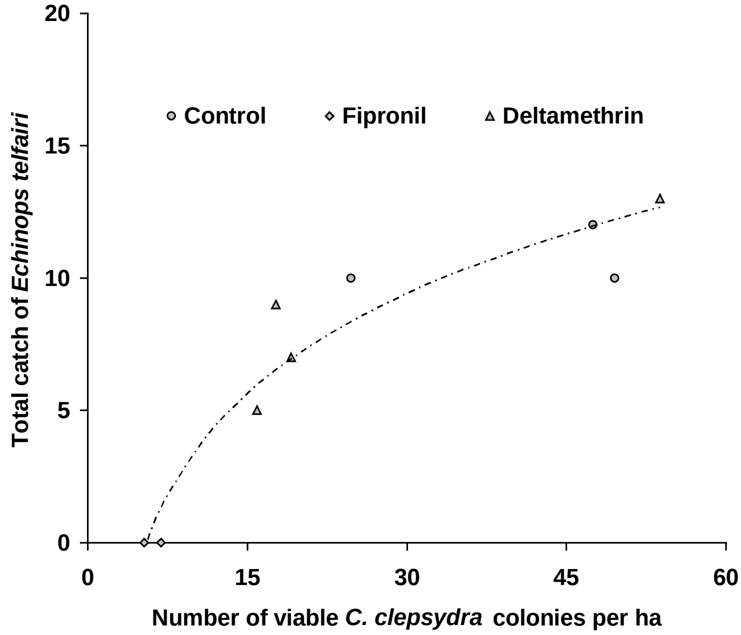

Figure 12 Relationship between viable *Coarctotermes clepsydra* colony density and the relative abundance of the lesser hedgehog tenrec, *Echinops telfairi*. Data are sums of three consecutive samples 16-24 weeks post-spray. Recaptured animals were only counted once.

These findings correspond well with the established food preferences. *E. telfairi* – along with *G. aurita* – showed the strongest preference for *C. clepsydra*, followed by *M. elegans* whose diet was dominated by termites. However, *M. elegans* appeared to be more flexible than tenrecs in that they consumed a wider range of termite species. *C. madagascariensis*, on the other hand, was more dependent on ants and beetles, and a relationship between the density of viable *C. clepsydra* colonies and the density of *C. madagascariensis* would only be expected if the density of ants was related to the density of *C. clepsydra* as well.

We did not test the relationship between the density of termite mounds and the density of the large-eared tenrec, *G. aurita*, and other insectivores because overall catches at the end of the dry season (16-24 weeks post-treatment) were too low (25 in *G. aurita* compared to 66 in *E. telfairi*). *G. aurita*, whose diet is mainly composed of termites (Glow and Venues, 1994), is a relatively rare tenrec species (Grime, 1988), and the number caught in the control was nearly as low as that in the fipronil treatment.

6.5 Fire impact

It has been mentioned that most experimental plots were affected by savanna fires (Photo 8). The first fire occurred in the deltamethrin plot in block 1 between 8 and 16 weeks post-treatment. This was the only plot which was completely burned. The remaining plots were affected in parts, usually between 16 and 22 weeks post-treatment. Sweep net samples were therefore taken in patches of unburned grassland if possible, but the position of drift fences and traps was not changed since the general pattern of burned and unburned patches was similar among plots.

The destructiveness and ecological consequences of savanna fires depend on the temperatures achieved. A high biomass and low water content of the standing crop favors hot and destructive fires (Goldman, 1990). Therefore, fires late in the dry season are usually more destructive in terms of the total area burned than early fires. On the other hand, young plants are still actively growing when attacked by early fires and are, therefore, more vulnerable (Bathwater, 1996).

Vegetation-dwelling arthropods are most severely affected, whether directly killed or deprived of their natural habitat. On the other hand, fire also leads to the rapid regret of burned tussocks of perennial grasses. Green leaves usually reappear after 1-2 weeks and the new grass cover is rapidly re-colonized from surrounding unburned patches. Grasshoppers are among the first pioneers exploiting the fresh food source (Duran ton et al., 1982). Some arthropods, including lepidopteron, may even get more abundant in burned sites, taking advantage of nutritious canopy regret after intense late fires (Bathwater, 1996).

The susceptibility of epigeal arthropods to savanna fire appears to be variable. Duranton et al. (1982) report a decline of carbide beetles but an increase of other coleopterans. Some arthropods hide themselves in burrows and crevices, others are nocturnal and are therefore not exposed at all. As far as termites are concerned, *C. clepsydra* is not negatively affected by fire (Caching, 1949).

Insectivorous vertebrates are indirectly affected through diminution of their invertebrate prey. In the present study, the fire impact can be considered as superimposed on the pesticide impact, resulting in a further depletion of the invertebrate food stock.

Chapter 7:
Impact on Freshwater Shrimp

Deltamethrin and fipronil caused massive kills in freshwater shrimp (Decapods: Caridea, Atyidae; Photo 9; Peveling, 2000). While the high toxicity of pyrethroids to crustaceans, aquatic insects and fish is well known (Eisler, 1992), the acute toxicity of fipronil came as a surprise. Until now, fipronil has been rated as one of the least toxic pesticides to aquatic fauna, even when applied at dosages higher than those applied in Malaimbandy (FAO, 1998; Laar, 1998, 2000). This misjudgment with respect to shrimp is due to the fact that laboratory or field toxicity tests are usually done with branchiopod test and monitor organisms such as water flea, *Daphnia magna*, or fairy shrimp, *Streptocephalus sudanicus*. These species appear to be less sensitive to fipronil than decapod shrimp (Peveling, 2000). It follows that the risk of pesticides to the latter cannot be predicted on the basis of branchiopod toxicity data.

To assess possible long-term effects on freshwater shrimp, we surveyed temporary or permanent water bodies in four plots. Four to five timed catches (1 min) were conducted to collect shrimp, using a push-net (mesh width 2 mm) measuring 34 cm (base) by 16 cm (radius of semi-circle). In addition, we determined chemical and physical baseline parameters, using a conductivity measuring probe and a Merck® field-kit for chemical analyses.

There was no evidence of long-term adverse effects on shrimp (Table 5). The observed abundance of freshwater shrimp was as variable within than between treatments. This was probably due to differences in the microclimate and the age of the ponds, i.e. the time elapsed since they were filled up again with water and a new development cycle was started. Even though our monitoring was only preliminary, we feel safe to conclude that effects on shrimp were transitory. Nonetheless, the toxicity of either insecticide to aquatic fauna has to be accounted for to avoid suppression of natural or commercial (shrimp farms) stocks. Moreover, side-effects on aquatic insects and fish should also be considered.

Table 5 Characteristics of ponds in Malaimbandy and relative abundance of freshwater shrimp

Treatment and block	Fipronil 1	Fipronil 2	Deltamethrin 3	Control 3
Date	**6.11.**	**9.11.**	**14.11.**	**13.11.**
Type	**Ponds**	**Ponds**	**Stream / ponds**	**Ponds**
Phys. & chem. parameters				
Conductivity (µS)	129	179	-	-
Temperature (°C)	24.2	24.6	26.6	-
pH	7.5	7.5	8	-
NH_4 (mg/l)	0	0.3	0.1	-
NO_2 (mg/l)	0	0	0	-
NO_3 (mg/l)	0	0-1	0	-
PO_4 (mg/l)	0	0	0	-
Carbonate hardness (mmol/l)	1.0	1.5	3.5	-
O_2 (mg/l)	5.5	2.8	8.5	-
Shrimp abundance (1-4 ponds)				
Median # / min (range) [surface and depth of pond]	2 (0-3) (9 m^2, 35 cm)	30 (20-35) [8 m^2, 50 cm]	4 (0-8) [14 m^2, 80 cm]	2 (0-6) [16 m^2, 80 cm]
„	1 (0-1) (5 m^2, 70 cm)	0 (0-2) [6 m^2, 60 cm]	10 (5-20) [12 m^2, 60 cm]	5 (0-7) [8 m^2, 70 cm]
„	100 (100-200) [9 m^2, 70 cm]	-	-	-
„	0 (0-1) [6 m^2, 60 cm]	-	-	-

Chapter 8:
Conclusions

This study revealed hazards of fipronil to terrestrial non-target organisms rarely encountered or reported in the recent history of locust control in Madagascar and elsewhere in the world. In a time when integrated and biological control techniques have become the basis of modern pest management, a fatal disruption of the food chain which eventually leads to the suppression or local extinction of insectivorous vertebrates does not only appear to be anachronistic but is certainly not compatible with present-day environmental standards.

In Latin America, fipronil has proved one of the most effective agents against mound-building termites – which are sometimes considered as pests of pastures (Pearce, 1997) – yielding about 100 percent control (Valerio et al., 1998). Our results do not only substantiate the extraordinary toxicity of fipronil to termites, but provide firm evidence of the importance of one species, *Coarctotermes clepsydra,* to the functioning and preservation of the food web, and underline the status of this species as a keystone taxon. Ironically, in the forties, when the ecological importance of insects was not yet valued and when DDT was welcomed as the ultimate tool to control invertebrate pests, *C. clepsydra* was also regarded a pest of cropland in Madagascar (Cachan, 1949). However, even then it was understood that this species was an important natural resource exploited by the rural population to feed their poultry.

For insectivorous animals living in semi-arid ecosystems, the dry season is the most critical period of the year. Food and water is in short supply and survival under extreme climatic conditions requires particular physiological and behavioral adaptations. Many insectivores rely on termites as a “staple food” since these are active even during dry periods (Deshmuk, 1986; Pearce, 1997). This is reflected, among others, in distinct seasonal changes in the invertebrate diet. For example, the proportion of termites in the diet of the lizard *Acanthodactylus dumerili* (Lacertidae) was 51 percent during the dry season, but only 6 percent during the rainy season (Cissé and Karns, 1977). It can, therefore, be concluded that late rainy season reductions of termite populations would have severer consequences for termite-feeders than early season reductions, since no alternative prey would be available. The pesticide treatments in the present study were conducted in May, i.e. at the beginning of the dry season in Western Madagascar, creating a situation similar to the scenario outlined above. In this respect, the dramatic effects on insectivores were the result of three factors: (a) type of insecticide (fipronil), (b) mode of application (full cover) and (c) time of treatment.

None of the vertebrates affected by fipronil in Malaimbandy is actually in danger of extinction in Madagascar. *M. elegans* and *C. madagascariensis* as well as *E. telfairi* are widely distributed in the southwestern parts of the island (Glaw and Vences, 1994). Thus it is unlikely that total populations were threatened by locust control. However, on a local scale, in areas subjected to intense treatments with fipronil, a suppression and/or reduced biological fitness of these species is possible. Moreover, predators of small vertebrates such as birds of prey and serpents are expected to be affected as well. Literally speaking, effects on the food chain translate into a “chain of hazard”. One indication derives from our study: the lesser hedgehog tenrec appeared to be affected by both the lack of termite and *M elegans* prey. Moreover, following the concept of bioindication (Römbke and Moltmann, 2000), it can be inferred from the effects on lizards and lesser hedgehog tenrec that rare insectivores are equally or even more vulnerable, in particular if they are specialized on termites.

Our findings may have important implications for the future of migratory locust control in Madagascar. The magnitude and duration of adverse effects of fipronil on vertebrate populations requires that this molecule must no longer be used as a full cover spray, and that its use as a barrier spray be suspended unless proof can be given that the environmental hazards are transient, i.e. that affected communities can recover from the hazards incurred. While the impact observed in our study may be unique to the Malagasy situation, it is possible that similar processes occur in other semi-arid ecosystems. It is, therefore, suggested to revise the environmental compatibility of this molecule on a global scale and to bring the prospects of and limits to its future use into new perspective.

Deltamethrin, on the other hand, did not appear to have a lasting effect on the food web and can be considered as more environmentally friendly, even though the acute toxicity to invertebrates was similar to fipronil.

The appraisal put forward in this report is mainly based on the observed hazards of fipronil to termites and vertebrates. Non-target effects of fipronil and deltamethrin on arthropods other than termites, including aquatic macro-invertebrates, certainly deserve an equally profound and critical assessment. This, however, is beyond the scope of the present report.

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Appendix I:

Photographs

Appendix I. Photographs

Photo 1. Mound of the termite *Coarctotermes clepsydra* with a basking *Oplurus cyclurus* (Iguanidae).

Photo 2. Hole 10 cm in diameter and 20 cm deep to measure the activity of *C. clepsydra*. The perforation indicates that the colony size is reduced, or that the colony is extinct.

Photo 3. Hole completely sealed by a healthy colony after three days to the level of the mound surface.

Photo 4. Colony of *C. clepsydra* killed by fipronil. The perforation of the mound is a typical sign of unhealthy or extinct colonies. (close-up view in Photo 2)

Photo 5. *Chalarodon madagascariensis* (Iguanidae)

Photo 6. *Mabuya elegans* (Scincidae)

Photo 7. *Echinops telfairi*, the lesser hedgehog tenrec (Tenrecidae)

Photo 8. Savanna fire in the vicinity of plot deltamethrin 3a, November 2000

Photo 9. Dead freshwater shrimp in a pond exposed to fipronil

Appendix II:

Sampling Methods

The principal sampling and monitoring methods employed in the present study are similar to those practiced in previous environmental surveys under the auspices of ONE (ONE, 1999) and are outlined in Table 6 (for details see the final report of the University of Antananarivo).

Table 6 Sampling and monitoring techniques used in the environmental survey in Malaimbandy

Taxon	Sampling/monitoring technique	# of subsamples/plot	Endpoint
Ground-dwelling arthropods [1]	Pitfall traps (plastic cups) lined up along barriers (drift fences) of plastic sheet (100 m)	3 drift fences	Relative abundance
Grass-dwelling arthropods [1]	Sweep netting along transects (100 m)	3 transects	Relative abundance
Flying insects [1]	Malaise traps	3 traps	Relative abundance
Termites [1]	Destruction (holes about 10-15 cm in diameter) of termite mounds	20 mounds	Repairing activity; colony survival
Small mammals	Sherman traps	15 traps; 2 checks per day	Relative abundance [2]
Reptiles	Visual counts (sightings) along fixed line transects (1 km)	3 transects; 2 counts per day	Relative abundance
	Pitfall traps (buckets) lined up along barriers (drift fences) of plastic sheet (150 m)	3 drift fences; 2 checks per day	Relative abundance [2]

[1] The identification and enumeration of specimens was carried out at the University. [2] Captured animals were identified, marked (toe clipping) and released. The mark-release-recapture method can be used to estimate absolute population densities. However, it requires sampling periods longer than in the present study (≥ 5 days).

Appendix III:
Data Analysis

Sweep-net catches of three consecutive days per sampling date were pooled, yielding a single datum for each subplot and plot, similar to pitfall and Malaise[8] trap catches. In reptiles (*M. elegans* and *C. madagascariensis*), the maximum number of sightings per transect of six individual counts during three consecutive days was used. Arthropod and reptile data were subjected to the same statistical analysis.

Catch or sighting data were normalized using a log (n+1) transformation. We then calculated the difference post- minus pre-treatment for each subplot or transect and sampling date. These derived values correspond to the "index of change (Δ D)" used by Norelius and Lockwood (1999) – except for replacement of the arcsine transformation of post/pre-spray proportions with the log (n+1) transformation – and describe the change in relative abundance compared to pre-spay levels.

A full factorial analysis of variance (ANOVA) was performed on these Δ D data. The Treatment mean squares (2 degrees of freedom, d.f.) were tested over the error mean square (d.f. = 19). Block effects were also included in the analysis but are not considered here. Multiple comparisons of means of Δ D were conducted with a Sidak test.

The effect E of pesticides is usually expressed as the percent change in relative abundance corrected for natural (= control) fluctuations (Peveling et al., 1999). E provides a concrete measure of the relative magnitude of pesticide-induced population reductions and is used to classify the risk to non-target invertebrates (OPPO, 1994). The effect was calculated according to:

$$E \text{ (percent)} = 100 \cdot (1 - C_b \cdot T_a / C_a \cdot T_b)$$

where C_b and T_b are the mean catch or sighting per plot *before* treatment in the control (C_b) and treatment (T_b), and C_a and T_a the mean catch or sighting *after* treatment in the control (C_a) and treatment (T_a). Significance of E was assumed if the respective differences in Δ D between control and treatment were significant. One such calculation was performed for each sampling period.

[8] Results for flying insects are not included in this report. For these, the reader is referred to the final report of the University of Antananarivo.

Data on lesser hedgehog tenures (which did not include pre-spray observations) were log (n+1)-transformed as explained above and analyzed with an one-way ANOVA. The Side test was used for multiple comparisons of means.

Statistical analyses of reptile diets were based on contingency tables with the categories (1) *treatment* versus *preferred prey* (taxon comprising the highest proportion of all invertebrate prey) and (2) *reptile species* versus *preferred prey*. (1) was analyzed with a χ^2-test and (2) with a log-linear model (G-test; Social and Rolf, 1995).

Proportional data from the study on *C. clepsydra* survival and behavior were arcsine-transformed. One-way ANOVA followed by Side multiple comparisons of means were performed on these transformed data.

Appendix IV:

Treatment Data

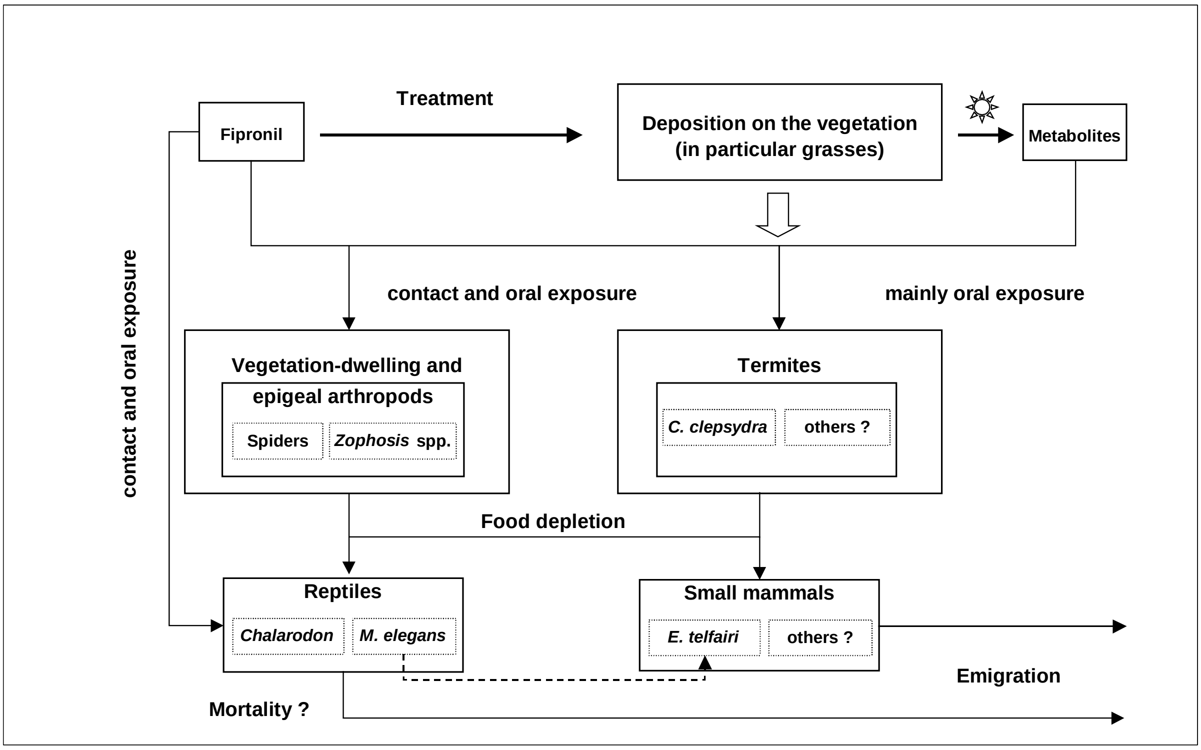
	Block 1		**Block 2**		**Block 3**	
	Deltamethrin	**Fipronil**	**Deltamethrin**	**Fipronil**	**Deltameth. a**	**Deltameth. b**
Date of treatment	15.5.2000		22.5.2000		31.5.2000	
Time of treatment	15.40-15.49	15.00-15.10	11.26-11.36	13.36-13.45	9.35-9.44	10.48-10.58
Aircraft	PA 36		Turbothrush Z-WSD [1]		Helicopter HU 500	
Sprayer	Micronair AU 4000		Micronair		Micronair AU 5000	
No. of atomizers	4		4		4	
Blade angle	∅		∅		35°	
Emission height (m)	12-20 (in valleys)		10-30 (in valleys)		10-30 (in valleys)	8-15
Track spacing (m)	> 100 m		100		> 100 m	
Number of tracks	8	9	11	11.5	9	9
Flow rate (l/min)	∅	∅	29	33	∅	∅
Sprayer RPM	∅	∅	∅	∅	5000	5000
Average forward speed (km/h)	∅	∅	202	200	∅	∅
Volume application rate (l/ha)	0.8	0.8	0.86	1	0.8	0.8
Dosage (g a.i./ha) a) nominal b) actual	 15 14	 4 3.2	 15 15	 4 4	 15 14	 15 14
Area sprayed (ha)	≈ 100	≈ 100	151	107	≈ 100	≈ 100
Relative humidity	∅	∅	50%	50%	∅	∅
Temperature °C (start-finish)	29-27	31-30	29-31	31-30	29-31.5	27-29
Wind direction	W	N	W	W	W	W
Wind speed (m/sec)	2-3	2-3	2-3	2-3	3	3
Cloud cover (%)	10	5	20	0	5	0
Formulation	17.5 UL	4 UL	17.5 UL	4 UL	17.5 UL	17.5 UL
Production date	6-8/99	4-6/98	6-8/99	4-6/98	6-8/99	6-8/99
Remarks			Runs longer than intended	Wind unstable in 2 runs		

[1] This aircraft was equipped with differential GPS (track guidance system); ∅ = missing data

Appendix V:

Routes of Fipronil Exposure and Effect

(Chart shown during the final presentation on November 20)



The flow chart shows principal exposure routes and effects of fipronil which eventually lead to a reduction in the density of insectivorous vertebrates. Vegetation-dwelling and epigeal arthropods are strongly reduced due to the contact and/or oral toxicity of fipronil, whereas termites are mainly exposed by collecting and feeding on contaminated grass. The grass contains residues of the parent compound and the desulfinyl photometabolite formed under solar radiation. The decrease of vegetation-dwelling and epigeal arthropods such as spiders and *Zophosis* spp. in combination with the strong decline of *Coarctotermes clepsydra* and, possibly, other termites cause an overall food depletion which in turn lowers the carrying capacity for insectivorous vertebrates. Effects on *E. telfairi* may be particularly severe due to the strong decline of both potential invertebrate (termites) and vertebrate (*M. elegans*) prey. It is implied that “starved” individuals can migrate into untreated areas since treatment plots were relatively small (100 ha). However, in case of large-scale treatments, net population reductions are expected due to increased intra- and interspecific competition and predation pressure.

Direct toxicity to reptiles cannot be ruled out with certainty according to previous reports (ONE, 1999). However, the present study does not provide any supporting evidence until now [9].

[9] Residue analyses for reptiles are pending. The interpretation put forward in this paragraph may have to be revised once the results are available.

Appendix VI:

Itinerary

May-June 2000

05/10/00	Travel Basle-Paris-Antananarivo
05/11/00	Meet Christian Ellwood and Frank Hawkins, IRG/PAGE; meet Wolfram Zehrer, plant protection specialist, and Gabriel R. Rasamoelina, *Directeur de la Protection des Végétaux*; meet Michel Lecoq, acridologist (= locust specialist), Director of CIRAD (*Centre de Coopération Internationale en Recherche Agronomique pour le Développement*) and FAO consultant in Madagascar
05/12/00	Meet Robert LeBlanc, IRG/PAGE; study relevant publications and reports on Environmental Impact Assessment collected by PAGE; second visit to the *Direction de la Protection des Végétaux*
05/13/00	Study final report of ONE on the ecotoxicological field work in Madagascar from 1998-1999; check and revise statistical analysis of some of the data
05/14/00	Study final report of ONE and other relevant documents
05/15/00	Meet Harizo Rasolomanana (ONE) and elaborate preliminary field work program; meet Philip DeCosse and discuss details of the project; meet General Victor Ramahatra, Director of the *Comité National de Lutte Antiacridienne* (CNLA), the institution responsible for locust control operations, including the experimental treatments of ONE; brief Rakotoary Jean Chrysostome, *Directeur Général Adjoint* of ONE, on the objectives and realization of the project
05/16/00	Elaborate detailed program for the field work in Malaimbandy and prepare for the trip
05/17/00	Travel to Malaimbandy; stop-over at the CNLA base in Miandrivazo; present objectives of the environmental monitoring, plan and coordinate the treatment in block II and check pesticide stocks
05/18/00	Revise experimental set-up; begin pre-spray observation in block II; accompany field researchers during their field work (deltamethrin plot); mark coordinates of plots, traps and observation transects
05/19/00	Accompany field researchers during their field work (fipronil and control plots) mark coordinates of plots, traps and observation transects
05/20/00	Revise and improve field monitoring methods; present and test new endpoints; produce preliminary maps of plots in block II
05/21/00	End pre-spray sampling in block II; demarcate new boundaries of treatment plots, put up flags for spray aircraft and install oil-sensitive papers to monitor droplet deposition and quality of treatment
05/22/00	Treatment of block II (deltamethrin and fipronil); analyze droplet deposition
05/23/00	Begin post-spray monitoring in block II; assess acute toxicity to aquatic and hydrophilous fauna
05/24/00	Assess acute toxicity to aquatic and hydrophilous fauna in treated plots; carry out similar assessments in control
05/25/00	Survey the area between Malaimbandy and Mahab to identify a third experimental block; mark out treatment and control plots
05/26/00	End post-spray monitoring in block II; mark out and put up sampling devices in block III (treatment plots)
05/27/00	Continue to mark out and put up sampling devices in block III (control); prepare final maps for blocks II and III; start pre-spray monitoring in block III
05/28/00	Travel to Antananarivo; stop-over at the CNLA base in Miandrivazo; plan and coordinate the treatment in block III
05/29/00	Inform F. Solofo Andiatsarafara*, Chef du Cellule Appui Scientifique aux Politiques*

Environnementales of ONE, about the project progress and arrange for a back-up from ONE during the last treatment; meet General Victor Ramahatra of CNLA to seek support for and coordinate treatment in block III; meet Wolfram Zehrer, consultant conducting an EIA of locust control ordered for the African Development Bank

05/30/00	Travel from Antananarivo-Paris-Basle

October-November 2000

10/25/00	Travel Basle-Paris-Antananarivo
10/26/00	Meet Harizo Rasolomanana (ONE) and Evah Andriamboavonjy (IRG/PAGE) and elaborate schedule for field work; meet Wolfram Zehrer, plant protection specialist, and Gabriel R. Rasamoelina, *Directeur de la Protection des Végétaux*
10/27/00	Travel to Malaimbandy; join environmental monitoring team (EMT)
10/28/00	Check progress of field work in block III together with EMT (22 weeks post-treatment); meet film team of CNLA
10/29/00	Conduct methodological study on termite activity (block III)
10/30/00	Re-evaluate termite activities (colony health) together with EMT
10/31/00	Revise termite activity classification system
11/01/00	Conduct preliminary analysis of the diet of reptiles
11/02/00	Start final monitoring of *Coarctotermes clepsydra* colony survival and viability in block I
11/03/00	Continue final monitoring of *C. clepsydra* survival and viability; analyze diet of *C. madagascariensis* and *M. elegans* in block I [10]
11/04/00	Continue diet analysis
11/05/00	Terminate final monitoring of *C. clepsydra* survival and viability; assess *C. clepsydra* colony density in block I; terminate diet analysis
11/06/00	Survey shrimp abundance in the fipronil plot, block I, and conduct chemical analyses
11/07/00	Start final monitoring of *Coarctotermes clepsydra* colony survival and viability in block II
11/08/00	Analyze data together with EMT; analyze diet of *C. madagascariensis* and *M. elegans* in block II
11/09/00	Survey shrimp abundance in the fipronil plot, block II, and conduct chemical analyses; continue diet analysis
11/10/00	Terminate final monitoring of *C. clepsydra* survival and viability; assess *C. clepsydra* colony density in block II; survey shrimp abundance in the control plot, block II; terminate diet analysis
11/11/00	Analyze data together with EMT
11/12/00	Start final monitoring of *Coarctotermes clepsydra* colony survival and viability in block III
11/13/00	Analyze data together with EMT; analyze diet of *C. madagascariensis* and *M. elegans* in block III
11/14/00	Survey shrimp abundance in the deltamethrin plot A, block III, and conduct chemical analyses; continue diet analysis
11/15/00	Terminate final monitoring of *C. clepsydra* survival and viability; assess *C. clepsydra* colony density in block III; visit of Evah Andriamboavonjy and collaborators (PAGE): visit field sites and present environmental monitoring techniques
11/16/00	Terminate diet analysis; hold final meeting with EMT; leave Malaimbandy to Miandrivazo (overnight stay)

[10] Stomach content samples containing termites were taken to Basle and re-analyzed there from December 4-15 to distinguish the different termite species.

11/17/00 Travel form Miandrivazo to Antananarivo

11/18/00 Prepare for presentation of preliminary results (data analysis, preparation of graphs, etc.)

11/19/00 Prepare for presentation of preliminary results together with EMT

11/20/00 Presentation of preliminary results together with senior researchers of EMT (meeting at the ONE head-office with participants from ONE, the University of Antananarivo, the Ministry of Agriculture, CNLA, IRG/PAGE and USAID); hold final meeting with EMT and ONE; visit IRG/PAGE and *Protection des Végétaux*

11/21/00 Travel from Antananarivo-Paris-Basle