PLANT PROTECTION

STUDY ON TITS FROM VELT/VOGELBESCHERMING VLAANDEREN

Analysis by FPS/Pesticide Service

Federal Public Service **Health, Food Chain Safety and Environment**
Service Plant protection products and Fertilizers

CONTACT

FPS Health, Food Chain Safety and Environment
Service Plant protection products and Fertilizers
Eurostation II, Place Victor Horta 40 box10
1060 Brussels
BELGIUM

Web: phytoweb.be
Tel.: +32 (0)2 524 97 97 (callcentre FPS)
Email: phytoweb@health.belgium.be
Contact form: phytoweb.be/en/contact

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Introduction

The triggers for performing this study were high numbers of dead nests of great tit and blue tit, and a suspicion of a link with pesticide use against the boxwood moth.

The test setup was meant to answer the following questions: *From which pesticides do we find residues in nests of great tits and blue tits? Are there any substances involved that are used to protect boxwood against moths? Which concentrations of pesticides can be found?*

An analysis has been performed on 7 nests originating from woods, since those spots are considered pesticide free. These nests served as a negative control. A total number of 1101 nests were brought in by individuals. Of those, 95 have been analysed for the presence of pesticides. These 95 nests were selected based on an indication of suspicion of pesticide use in the direct neighborhood.

Much information can already be found in the VELT study. The only complementary data that were available for the Service Plant protection products and fertilisers are the complete results from the analysis of the concentrations of substances in each nest. The exact numbers of active substances from plant protection products in each nest could in this way be considered. The measured concentrations have also been compared with chronic toxicity endpoints (NOAEL, No Observed Adverse Effect Level), while the original study only considers LD_{50}-values (acute toxicity, based on Lethal Dose for 50% of the organisms). Mixture toxicity has also been assessed, for nests with several measured active substances.

Evaluation of the study results

Numbers of active substances

Forests:

7 nests were analysed. Three nests are free of residues, 4 contain residues of DDT. Range: 0.004 – 0.01 ppm, mean: 0.007 ppm. Apart from DDT no active substances from plant protection products were found.

Gardens:

On a total of 95 nests there are 8 nests (8%) that contain 4 or more active substances from plant protections products. Or DDT and 3 or more active substances, since DDT is always present in these nests. In the majority of nests only DDT has been found. In nearly all nest where more than 1 active substance was found, DDT was present. In the majority of the nests a presumed toxicity can only be caused by DDT, since no other substances were found. It seems unlikely however that exposure of tits to DDT was higher last year than previous years, considering the non-approval of DDT for many years and historical contamination. Due to this non approval, no action on normative level is possible for DDT.

Table 1: numbers of active substances per nest

Number of active substances	Number of nests	Remarks
8	1	DDT present
6	1	DDT present
5	2	DDT always present
4	4	DDT always present
3	10	DDT always present
2	22	Of which 21x DDT
1	51	always DDT
No active substances	4	
only DDT	51	

For the analysis a selection was made for nests coming from gardens where there was a suspicion of use of pesticides. In this way however, a distorted image can be obtained. For a total of 1101 nests presented only 95 nests were analysed. It is likely that less substances and lower concentrations could have been found in other nests.

Overall, 36 active substances have been found. Of those, 23 were only found in one single nest. In 8 nests on a total of 95, 4 or more active substances have been found, amongst which always DDT. The number of nests with multiple active substances is relatively low, certainly when making abstraction of DDT.

Measured substances and concentrations

DDT: In 89 of 95 garden nests DDT has been found. Mean: 0.027 ppm. Range: 0.004 – 0.19 ppm (mg/kg). In the 7 negative control nests from forests, also 4 times DDT was found.

23 substances have been measured only once:

- Aclonifen
- Biphenyl
- Bixafen
- Chlorpropham
- Chloridazon
- DEET (repulsive/biocide)
- Dichlobenil
- Dinotefuran (neonicotinoid insecticide, a substance that has never been registered in Belgium as a plant protection product)
- Dodine
- Flufenacet
- Fluopyram
- Heptachlor (organochlor insecticide)
- Imazalil
- Isoproturon
- Isoxaben
- Methoprene
- Metobromuron
- Propamocarb
- Propoxur (not approved)
- Propyzamide
- Spinosad
- Spiroxamine
- Tebuconazole

Due to the low frequency of occurrence a conclusion concerning the effect of these substances is not possible.

Difenoconazole was detected 4 times as indicated in the VELT report, but each time below the limit of detection.

The 12 other substances have been measured several times:

- Imidacloprid (8x), mean: 0.017 ppm (0.005 – 0.0767 ppm)
- Piperonylbutoxide (8x), mean 0.022 ppm (0.0046 – 0.0776 ppm) (registered to fight boxwood moth). This is a synergist of pyrethrin insecticides, which have themselves not been found in the residue analysis. But this can be due to rapid degradation.
- Fipronil (7x), mean 0.073 ppm (0.01 – 0.34 ppm)

- Dieldrin (4x), mean 0.0098 ppm (0.006 – 0.018 ppm)(organochlor insecticide)
- Diflufenican (4x), mean 0.005 ppm (0.004-0.0069 ppm)
- Permethrin (4x), mean 0.08 ppm (0.018 – 0.2296 ppm)
- Captan (3x), mean 0.62 ppm (0.1-1.7 ppm)
- Indoxacarb (3x), mean 0.025 ppm (0.0139 – 0.0332 ppm) (registered to use against boxwood moth)(found 1 x with only DDT, 1 x with imidacloprid+DDT and 1x with piperonylbutoxide+DDT)
- Prosulfocarb (3x), mean 0.029 ppm
- Tetraconazole (3x), mean 0.0038 ppm (0.0036 – 0.0041 ppm)
- Propiconazole (2x), mean 0.007 ppm
- Pyrimethanil (2x), mean 0.020 ppm

For all substances that were found multiple times, the chronic toxicity has been considered in Table 2.

Risk assessment per single substance

In the VELT study a comparison has been made between the measured concentrations and the acute toxicity values – doses where mortality has been observed for 50% of tested animals in studies. The measured concentrations can also be compared with chronic toxicity values which are data that are available for active substances of plant protection products. In a chronic toxicity study a NOAEL is determined, which is a dose where no effects have been observed. As indicated in the VELT study, the results from chronic toxicity tests have not been obtained with tits, but with quails or ducks. In these studies adult birds are exposed during a number of weeks to the tested substance which is mixed into their diet. After that, egg-laying is observed, as well as hatching and survival of the chicks for 14 days. Although this study is performed with different bird species, the study set up and study results give information about the effects of a substance on the reproduction of birds. These chronic toxicity values are therefore relevant in the framework of this study.

A residue analyses was only done for the entire chicks. A toxicological assessment has not been performed. This makes it more difficult to evaluate the cause of death, and potentially link it with the presence of residues.

Table 2: active substances found several times with their toxicity values and calculated TER (toxicity Exposure Ratio)

Substance	acute LD50 (mg/kg bw/d)*	chronic NOAEL (mg/kg bw/d)*	Highest measured concentration	acute TER	chronic TER
imidacloprid	503	9,3 (NOAEL)	0,077	6532,5	120,8
piperonylbutoxide (8x) (not a plant protection product)	>2250	27	0.0776	29000	348
fipronil	11,3	0,88	0,34	33,2	**2,6**
*Dieldrin***		not approved	0.018		
diflufenican	2150	91,84	0,0069	311594,2	13310,1
*Permethrin***	>9800	50 (not approved)	0,23	42608,7	217
captan	>2000	74,4	1,7	1176,5	43,8
indoxacarb	98	75,7	0,033	2969,7	2293,9
prosulfocarb	>2250	131	0,078	28846,2	1679,5
tetraconazole	132	1,6	0,0041	32195,1	390,2
*Propiconazole***	>2510	79	0,01	251000,0	7900
pyrimethanil	>2000	95,96	0,036	55555,6	2665,6

*: the toxicity endpoints used come from the Review Reports for the active substances at EU level.
**: these substances are not approved at EU level as a plant protection product active substance. Approved substances are considered more relevant here since only for those measures are possible. For dieldrin endpoints are lacking, but this substance has been withdrawn ('not approved') for a long time. Permethrin and propiconazole are however approved as active substances for biocides. Toxicological endpoints for permethrin come from the biocide assessment report.

Remark: For the registration of a plant protection product, a risk assessment is performed with a model where the estimated exposure of a bird for a specific application is compared with the acute or the chronic toxicity. A TER value (Toxicity Exposure Ratio) is calculated, where the

toxicity endpoint is divided by the expected exposure for a specific use of a plant protection product. The TERs have been calculated in the above table. The limit value of a TER is 10 for acute risk assessment and 5 for chronic risk assessment. As long as the TER is higher than the relevant value, risk is considered acceptable.

The chronic toxicity is determined in a study where birds are administered doses of a substance via their food. It is not possible to determine which doses the dead chicks of tits have ingested, we only know the concentrations that have been measured in the entire bodies. One can only presume that these measured concentrations are lower than the received total doses due to metabolization and excretion of some of the ingested substance. By lack of further data, both values (toxicity and measured concentrations) are being directly compared here in order to have an idea about potential risk. Although these TER-values are not conform to the guidance for the risk assessment for plant protection products, they still give an indication of the potential risk.

Only for fipronil an unacceptable TER is obtained in Table 2, and only for the highest measured value. Fipronil is not approved at EU level as a PPP active substance. Consequently there are no plant protection products registered in Belgium that contain fipronil.

Remark: in the meanwhile exposure of tits to pesticides via hair of cats and dogs (treated against fleas and ticks) used for making nests, has been assessed. Relevant exposure via this route is considered very likely (https://www.vilt.be/mezensterfte-te-wijten-aan-honden--en-kattenhaar).

Only 3 substances are registered to be used against boxwood moth: indoxacarb, piperonylbutoxide and spinosad. Only spinosad is not in Table 2, but this substance has only been measured once, and only traces were detected – a too low concentration for quantification. For indoxacarb the TER indicates a limited risk. Piperonylbutoxide is not an active substance under the plant protection products directive, but endpoints from the ECHA assessment report from January 2017 have been used to assess the risk. Based on this, limited risk is anticipated.

For sake of completeness, although both substances have only been measured in one single nest: for dodine and methoprene high concentrations have been measured. Dodine therefore considered in the table below.

substance	acute LD50	chronic NOAEL	Highest measured concentration	acute TER	chronic TER
dodine	917	200	0,394	2327,4	507,6

This indicates a limited risk for dodine.

Methoprene is not an approved active substance under the plant protection legislation.

Mixture toxicity

The combined risk for nests where several substances were found has been assessed. A selection of the most critical nests was made to do this assessment. Only active substances for which toxicity values are available have been taken into account. These are substances which have recently been reviewed for approval in the EU as active substance for plant protection products.

In a number of nests multiple substances have been measured, and the possibility of an accumulation of toxicity should be considered. Through a calculation assessment is possible of what is called 'combined toxicity' or 'mixture toxicity', where the toxicity provoked by individual substances is added together (formula for the calculation: $1/TER_{combi} = \sum(1/TER_i)$).

For a number of nests where this seemed relevant this mixture toxicity has been calculated. The nests were selected based on the high number of measured substances and/or high concentrations measured:

Nest: ME027

concentration	substance	chronic endpoint	TER	
0,071	captan	74,4	1047,887	
0,048	DDT	/		
0,006	dieldrin	/		
0,022	heptachlor	/		
0,004	spiroxamine	29,3	7325	
			TERmix:	916,7

Nest: ME048

substance	concentration	TER	1/TER
DDT	0,035	/	
fipronil	0,093	9,46	0,106
imazalil	0,0147	1632,7	0,00061
prosulfocarb	0,0038	34473,7	2,9E-05
			∑ = 0,1063
		TERmix:	9,40

Nest: ME057

concentration	substance	chronic endpoint	TER	
0,014	DDT	/		
0,005	dieldrin	/		
0,016	piperonylbutoxide	27	1687,5	
0,0251	isoxaben	67	2669,323	
0,0133	propyzamide	58,5	4398,496	
			TERmix:	837,1

Nest: ME059

concentration	substance	chronic endpoint	TER	
0,0142	aclonifen	141	9930	
0,013	DDT		/	
0,0046	piperonylbutoxide	27	5870	
0,009	flufenacet	9,87	1097	
0,0379	metobromuron	21,6	570	
0,078	prosulfocarb	131	1679	
			TERmix	283

Nest: ME084

substance	concentration	TER	1/TER
captan	1,7	43,8	0,023
DDT	0,005	/	0
difenoconazole	0,0472	205,7	0,0048
dodine	0,3935	508,3	0,0020
fluopyram	0,033	136,4	0,0073

propiconazole	0,009	8777	0,0001
pyrimethanil	0,0356	2695,5	0,00037
tebuconazole	0,031	187,1	0,0053
			∑ = 0,043
		TERmix:	23,41

Nest: ME099

concentration	**substance**	**chronic endpoint**	**TER**
0,007	DDT	/	
0,0277	DEET	/	
0,0187	imidacloprid	9,3	497
0,0305	propoxur	/	
		TERmix:	497

In the above calculations the mixture toxicity TER remains above the trigger value that is used for risk assessment for plant protection products. That trigger value is 5. Any TER above this value indicates an acceptable risk. The risk for the exposure to a series of measured substances in one nest seems acceptable, considering the limitations in the assessments as explained before. In nest ME048 it is mainly fipronil individually that is causing the toxicity. In nest ME084 the addition of many substances but all in low concentrations still shows a relatively low concern. To be remarked is that substances that are not approved for a long time under the plant protection legislation have not always been taken into account in the calculation. If the evaluation of the active substance was done recently, toxicological endpoints are however available, and were considered.

Nest: ME071	0,0036	chlorpropham	not approved TER: 27777
	0,009	DDT	not approved
	0,34	Fipronil	**TER: 2.6**
	0,1623	methoprene	not approved

TERmix: 2.6 (no influence from chlorpropham)

ME090		**TER**
0,055	DDT	not approved
0,2296	Permethrin	217 (not approved)
0	piperonylbutoxide	/

0,0036 tetraconazole 444,4
TERmix: 146

Nests ME071 and ME090 indicate that for many nests substances have been measured that are not or no longer approved as active substance in plant protection products in the EU. This limits the completeness of the calculation. Actions at the level of the approval of these substances in the framework of the plant protection products legislation are however not possible.

Conclusion

In the majority of dead nests only DDT has been found. Since this substance has been forbidden many years ago, it is not possible to take measures.

Based on an approximative comparison between measured concentrations of active substances with existing toxicity endpoints for birds there is for only one nest, ME071, an indication of an unacceptable risk due to a high concentration of fipronil. This substance is not approved at EU level as an active substance for plant protection products. Consequently there are no registered plant protection products that contain this active substance in Belgium. At level of the Service for Plant protection products and Fertilisers, no measures are therefore possible.

After comparison with the available acute and chronic toxicity endpoints, the other measured concentrations appear to give little indication of an unacceptable risk. This is the case even when mixture toxicity is taken into account for nests where several active substances were present. There are no sound indications that allow to link the mortality with effects caused by the presence of active substances, or with a combined toxicity effect from several active substances.

Overall, 36 active substances have been found. Of those, 23 were only found in one single nest. In 8 nests on a total of 95, 4 or more active substances have been found, amongst which always DDT. The analysed nests were selected based on an indication of a suspicion of use of plant protection products in the neighborhood. This means that the analysed nests may well be giving a more negative image then what can be expected from non-analysed nests from other surroundings. The number of nests with multiple active substances is relatively low, certainly when making abstraction of DDT.