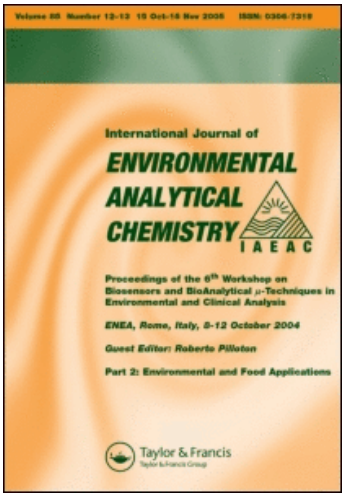

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Carnivorous Insects as Bioindicators of Environmental Contamination: Organochlorine Insecticide Residues Related to Insect Distribution in Terrestrial Ecosystems*

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The impact of organochlorinated insecticides on the relative contamination of ants and sapro-necrophagous and carnivorous beetles has been evaluated in various agricultural and forested areas in Belgium. A novel micromethod of organochlorinated insecticide extraction and clean-up at trace levels is described. This analytical procedure involves sample clean-up on Sep-Pak Florisil microcartridges and high resolution gas chromatography analysis with an automatic solid sampler. The efficiency of extraction, clean-up and gas chromatography analysis is discussed.

In spite of the legal interdiction of most organochlorine insecticides in this country (with the exception of lindane), residues of DDT and dieldrin were still detected in beetles and in ants. The contamination of insects was generally low (mean values ranged from 3 to 70 ppb according to the nature of insecticide) although a few samples contained high concentrations of prohibited insecticides (up to 274 ppb of dieldrin and 419 ppb of DDT metabolites). The authors suggest that insect contamination by organochlorine insecticide residues in forested areas would not result from a direct intoxication due to utilization of these xenobiotics on the sampling sites. Indeed, codistillation phenomenon conjugated with atmospheric transport of contaminated particles could induce environment contamination far away from the sites of insecticide applications in the direction of the West dominant winds in Belgium.

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The consequences of such a contamination of insects by organochlorine insecticide residues are discussed.

KEY WORDS: Lindane, dieldrin, DDT, ants, beetles, bioindicators.

INTRODUCTION

The rapid spread of chemical industry after the Second World War has widely contributed to environmental pollution by increasing amounts of persistent toxics such as organochlorinated pesticides. According to their high lipophility, their low solubility and their low biodegradability, these xenobiotics accumulate up food chains in terrestrial ecosystems.[1–3] In spite of the legal interdiction of most organochlorine insecticides in Belgium (with the exception of lindane) since 1974 and 1976, the environment appears to be particularly contamined by chlorinated hydrocarbon insecticide residues in this country.[4]

In terrestrial ecosystems, insects take an important part in the transport and in the reintegration of organic matters in soils. Indeed, carnivorous, necrophagous and detritivorous insects such as ground beetles, dung beetles and ants can change the physical and chemical soil properties. Moreover, ants live in nests the construction of which involves soil mixing and aeration as well as water penetration in water repellent soils.[5] Therefore, it is of the utmost interest to investigate the organochlorine pesticide accumulation in these carnivorous and detritivorous insects which belong to high trophic levels and are potential preys of insectivorous birds particularly sensitive to these persistent xenobiotics. The present paper deals with an estimation of the impact of field organochlorinated insecticide pulverisation on the relative contamination of ants and sapronecrophagous and carnivorous beetles, in various agricultural and forested areas in Belgium.

EXPERIMENTAL

Insect sampling and localization

Figure 1 shows the sampling places of ants and beetles. Sapronecrophagous and carnivorous beetles have been collected by means of

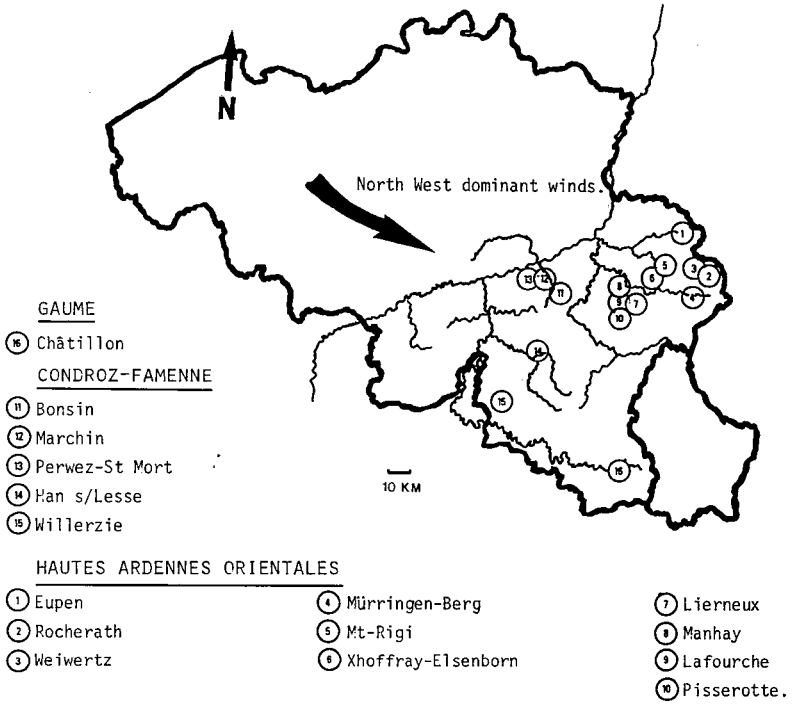

Figure 1 Localization of the ant and beetle sampling stations in agricultural (Gaume, Condroz-Famenne) and forested (Haute Ardenne Orientale) areas.

baited pitfall traps (Barber type) randomly distributed in 70 stations located in cultivated areas (Gaume and Condroz-Famenne) and in forested areas (Haute-Ardenne Orientale). The beetles trapped were essentially: *Abax ater, Harpalus sp, Chrysocarabus auronitens, Carabus violaceus, Carabus arvensis, Pterostichus madidus, Geotrupes stercorosus, Necrophorous vespilloïdes, Necrophorus humator, Silpha tristis, Silpha carinata* and *Oceoptoma thoracica.*

Ant workers have been collected in 65 nests located in agricultural areas (Condroz-Famenne: 3 stations) and in forested areas (Haute-Ardenne Orientale: 7 stations). The ant species so far collected were typical on one hand of agricultural areas, i.e. *Myrmica laevinodis, Myrmica ruginodis* and *Lasius niger*, and on the other hand of forested areas, i.e. *Formica polyctena* and *Formica rufa.*

Each sample of insects was frozen to death at −40°C and

weighed. All the beetle species of a sample were pooled and analysed together. Due to the small size of insect samples (100 to 500 mg fresh weight), there was a real need to develop an accurate and sensitive micromethod of organochlorinated insecticide extraction at trace levels. Pesticides were immediately extracted from the fresh material, or subsequently from frozen material preserved at −40°C.

Extraction procedure

All solvents (hexane, acetone, diethyl-ether) were HPLC and Pesticide grade. Analytical standards and solvents have been purchased from Alltech Belgium (Eke, Belgium). Samples were homogenized in 5 ml hexane-acetone (1/1; v/v) containing 1 g anhydrous Na_2SO_4 by means of an Ultra-Turrax homogenizer (Janke and Kunkel Gmbh, Ika werk, Staufen i.B., W. Germany). After centrifugation at 1750 g for 15 min (centrifugation I), the resulting pellet was treated similarly with 5 ml hexane-acetone mixture (centrifugation II). The supernatants from both centrifugations were pooled and evaporated just to dryness under a gentle stream of nitrogen. Dry extracts were immediately solubilized in 1 ml hexane.

Extraction efficiency has been tested by means of op'DDE (a DDT metabolite never found in biologic samples analysed) used as internal standard. A known amount of op'DDE was added to sample before extraction procedure and the percent of recovery was measured between the different extraction steps. Mean recovery of internal standard after two sample extractions (centrifugation I+II) was $93.2 \pm 8.75\%$. A further extraction of the resulting pellet (centrifugation III) leads only to a further $6.4 \pm 5\%$ recovery.

Sample clean-up

Analytical procedures for chlorinated pesticides analysis require efficient sample clean-up prior to the high performance capillary gas chromatography analysis. The adsorbent "Florisil" is commonly used in the clean-up step.[6–9] However, other widely used chlorinated industrial compounds, namely PCBs, have been identified at trace levels in a variety of animal species. Due to their ubiquity in the environment, PCBs have been under scrutiny to eliminate possible

interferences with organochlorine pesticides analysis. Since then, clean-up procedures have been proposed to separate chlorinated hydrocarbon from PCBs.[7–9] However, these sample preparations are time consuming and involve several elutions with large volume of various solvent mixture.

A rapid sample clean-up scheme is proposed as an alternative to those more tedious Florisil clean-up procedures. This rapid samples preparation technique uses Sep-Pak Florisil cartridges (Waters Associates, Inc. Milford, Massachusetts, USA). This technique, used in conjunction with high resolution capillary gas chromatography, provides rapid determination of organochlorinated pesticides. A Sep-Pak cartridge was attached to a 10 ml glass syringe with Luer end fitting and successively pre-eluted with 2 ml of diethyl-ether, diethyl-ether/*n*-hexane (1/1; v/v) and *n*-hexane. Sample solution (1 ml) was passed through the cartridge. Pesticides were eluted from the cartridge with two 3 ml portions of hexane while unwanted high polarity compounds were retained on the Sep-Pak. The several eluents (about 5 ml) were collected in 6 ml glass vials and evaporated up to 1 ml under a gentle stream of nitrogen.

Recovery efficiency

Comparative recovery efficiency of the organochlorine pesticides has been tested on Sep-Pak cartridges and on traditional Florisil columns.[7–9] For that purpose, 3 replications of extraction procedure and elution through the different types of columns have been performed with an organochlorine insecticide standard mixture (αBHC, HCB, lindane, aldrin, heptachlor, heptachlore epoxide, dieldrin, op′ and pp′DDD, op′ and pp′DDE and pp′DDT; 50 pg of each component/μl hexane). The data reported in Table I show that the mean recovery percentage reached about 100% for each of the 12 components taken into account after elution through the Sep-Pak Florisil cartridges. On the other hand, traditional column clean-up has resulted in poor reproducible recovery efficiency. Moreover, these procedures require large volume of solvent (up to 400 ml vs. 5 ml for Sep-Pak technique) and involve high concentration of extraction solvent. Then, these extensive sample preparations may increase analytical error and affect sample recovery. This is especially true when working with trace amounts of residues.

Table I Recovery efficiency (±standard deviation) of organochlorine insecticides after clean-up procedure on traditional Florisil columns and on Sep-Pak cartridges. Results are expressed in percent

Insecticide	E.P.A.[9]	Reynolds[7]	Sep-Pak cartridge
α BHC	129±9	98±7	106±2
HCB	63±8	46±37	104±7
Lindane	93±14	79±12	102±6
Heptachlor	150±16	103±36	101±3
H. epoxide	62±9	104±2	107±18
Aldrin	78±4	59±7	94±2
Dieldrin	56±6	110±3	109±5
op'DDE	54±18	125±24	97±3
pp'DDE	49±8	81±5	100±1
op'DDD	—	100±3	103±1
pp'DDD	—	91±2	120±11
pp'DDT	70±6	132±14	103±2

(—): not realised.

As a consequence, the pesticide clean-up method used eliminates lengthy sample preparation, minimizes analytical error and results in high recoveries. A typical result of Sep-Pak Florisil clean-up is shown in Figure 2 where one can see that the use of high resolution capillary gas chromatography results in a very good separation of organochlorine pesticides.

Gas chromatography analysis

Samples were analysed with a Carlo Erba Fractovap 4130 gas chromatograph equipped with a Ni^{63} ECD. The organochlorine pesticides were separated on a 25 m × 0.22 mm chemically bonded fused silica column coated with 0.12 μm of CP-Sil 8 CB liquid phase (Chrompack, Antwerpen, Belgium). Carrier gas was hydrogen at an inlet pressure of 0.5 bars (1 ml/min); make up gas was AR/CH_4 (90:10) at a flow rate of 30 ml/min; injector temperature was 260°C and detector temperature was 275°C. The gas chromatograph was equipped with an automatic solid sampler (Carlo Erba, Milano, Italy) mounted over the injection port on a tube through which the samples fall into the vaporizer of the split-splitless injector (split valve closed). Inside this sampler, a drum retained up to 24 samples contained in small glass capillaries with a maximum volume of

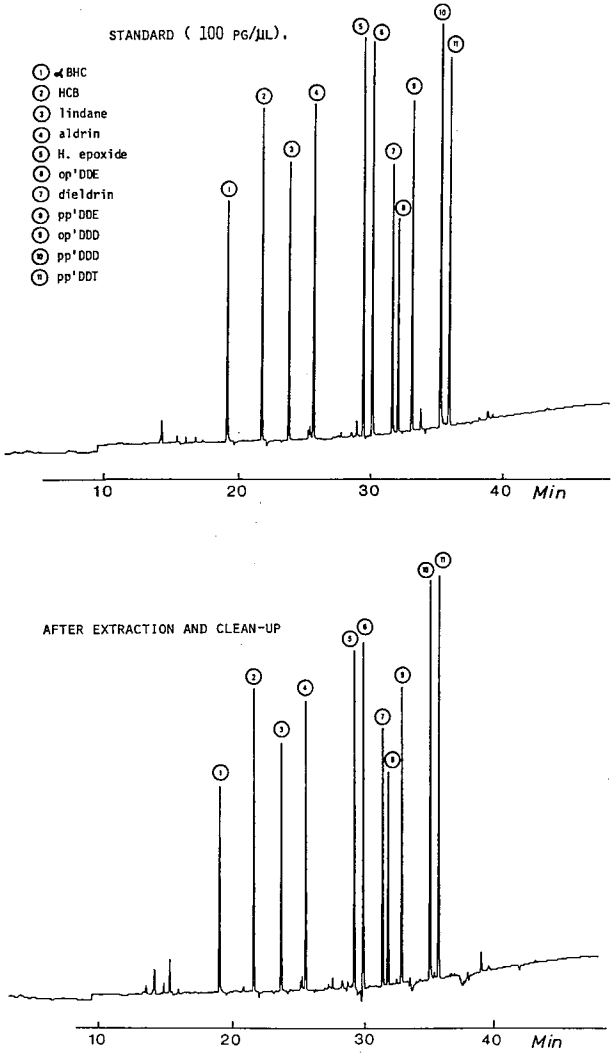

Figure 2 Recovery efficiency of organochlorine insecticide residues after extraction step and Sep-Pak clean-up procedure.

$2.16 \pm 0.13\,\mu l$ in air free conditions. These glass capillaries were prepared to receive samples by 3 successive flushes of hexane and acetone. Solvent was evaporated under a gentle stream of nitrogen and the inner surface of glass capillaries was coated with SE-30 (2% in *n*-hexane). These were filled with $2\,\mu l$ of the sample extracts in *n*-hexane with a Hamilton microsyringe ($5\,\mu l$). The solvent was evaporated at the end of the filling leaving the organochlorine residues as a dry film on the inner coated surface of the sample capillaries. These were placed into the corresponding slot of the solid sampler which is closed, purged with hydrogen carrier gas for 5 min and cooled down to $-4°C$ in order to retain the components with relatively high vapor pressure (e.g. lindane).

Reproducibility tests have been realised with pesticides standards ranged from 6 to $150\,\mu g/\mu l$. The recovery efficiency of the 12 organochlorine pesticides has been tested. For that purpose, automatic analysis of these mixtures have been performed as a function of remaining time in the solid sampler before injection and analysis (time ranged from 1 to 7 h). Mean recovery percentages reached up to $95 \pm 4.5\%$ for each of the components taken into account whatever the time the samples remained in the automatic solid sampler.

Sample injections were done at a column temperature of 80°C (initial hold = 1 min). Then, the column was programmed to 240°C at 4°C/min (final hold = 5 min). After the column was cooled to 80°C, the next sample was automatically injected. One cycle requires 1 h.

Quantitation of organochlorine insecticides

The peak areas were integrated with an electronic integrator LDC Milton Roy (Shannon Airport Co. Clare, Ireland) and the peaks were identified by retention time. The quantity of each insecticide was calculated from the added amount of op'DDE internal standard multiplied by the peak ratio of compounds of interest and internal standard.

RESULTS AND DISCUSSION

Chlorinated hydrocarbon insecticide residues were found in the majority of samples of beetles and ants. The principal residues

occurring in both groups of insects were lindane, dieldrin and DDT metabolites (especially pp'DDE). Only in beetles, little amounts of heptachlor and heptachlor epoxide have been detected. However, the level of beetle contamination by these residues was particularly low. Moreover, as far as the latter insecticides are concerned, no significant differences were found between the contamination degree of beetles trapped in agricultural and in forested areas (respectively: 1.7 ± 3.5 and 5 ± 8.9 ppb for H. epoxide and 3.1 ± 5.7 and 8.5 ± 18.4 ppb for heptachlor).

Contamination of beetles

The data reported in Table II clearly show that DDT residues were the most abundant in all the regions so far prospected in Belgium.

Table II Mean concentrations ($\pm$standard deviation) of organochlorine insecticide residues detected in beetles trapped in forested areas (Haute Ardenne Orientale) and in agricultural areas (Gaume + Condroz-Famenne). Results are expressed in ng of insecticide/g of beetles (fresh weight).

	Stations	Lindane	Dieldrin	$\sum$DDT (pp'DDE + DDD)
Gaume	Châtillon $n=12$	4.7 ± 7.7	30.8 ± 85.7	17.1 ± 32.4
Haute Ardenne Orientale	Weiwertz $n=4$	0.3 ± 0.6	—	0.1 ± 0.2
	Mürringen $n=4$	—	—	52.8 ± 64
	Berg $n=7$	16.1 ± 32.7	23.3 ± 47.4	67.4 ± 156
	Elsenborn $n=16$	5.3 ± 5.9	1.9 ± 4.9	22.6 ± 28.5
Condroz Famenne	Han s/Lesse $n=7$	8.6 ± 10.7	1.1 ± 2.2	18.8 ± 35.5
	Lierneux $n=3$	—	32.3 ± 56	18.7 ± 22
	Bonsin $n=4$	—	3.5 ± 7	16.3 ± 21
	Willerzie $n=9$	5.2 ± 8.9	1.1 ± 3	12.6 ± 32

(—): below the detection level (0.1 ppb).
$n=$: number of samples.

According to the trapping places, the mean concentrations of DDT ranged between 0.1 and 67 ppb. However, in each station, the variations from one sample to another were very wide (range: 0–419 ppb). All the other residues were significantly less abundant than DDT (Mann–Whitney: $P < 0.05$). A Kruskall–Wallis test realised on the residues of each insecticide in the beetle samples shows no significant difference between the contamination of beetles in the different trapping places ($P < 0.05$). Although the insects trapped in Hautes Ardennes Orientales appeared to be more polluted than those from other places only because a few samples were much more contaminated than the others. Indeed, standard deviations calculated for the mean concentrations of residues have usually very high values.

The most contaminated samples by lindane, dieldrin and DDT included essentially sapronecrophagous beetles: *Geotrupes stercorosus*, *Sipha tristis* and *Necrophorus vespilloïdes*.

Contamination of ants

From Table III, it can be seen that metabolites of DDT are generally the most abundant residues in ants with the exception of Manhay, Lafourche and Pisserotte, 3 stations located in forested areas. Interestingly, residues of dieldrin occurred only in ants collected in these 3 sampling places. In forested areas, mean concentrations of lindane ranged from 8 to 35 ppb with high variations in the contamination degree in each sampling place. A Krustall–Wallis test applied to the insecticide concentrations in ants as a function of the nest localization (forested areas *vs* cultivated fields) and of the species concerned shows no significant differences between the lindane and DDT contamination degree, on one hand of the ants belonging to the genus *Myrmica* and *Formica* and, on the other hand of ants collected in the different sampling places.

Contamination degree of ants and beetles in forested vs. agricultural areas

The data reported in Figure 3 were obtained from the compilation of the different data related to insecticide residues measured in the beetles and in the ants collected in forested areas and cultivated fields. From Figure 3, it can be concluded that:

Table III Mean concentrations (±standard deviation) of organochlorine residues detected in ants collected in Haute Ardenne Orientale and in Condroz–Famenne nests. Results are expressed in ng of insecticide/g of ants (fresh weight).

	Stations	Lindane	Dieldrin	∑ DDT (pp'DDE + DDD)
Haute Ardenne Orientale	Mt-Rigi $n=5$	34.4±15	—	45.9±43.6
	Rocherath $n=6$	34.6±44.6	—	43.4±26.5
	Eupen $n=13$	12 ±19.5	—	20.4±35.3
	Xhoffray $n=4$	8.8±15.4	—	34.6±61
	Manhay $n=3$	6.3±10.9	15 ±13	—
	Lafourche $n=7$	9.6±19.1	15.8±9.2	—
	Pisserotte $n=4$	8.5±9.9	5.4±10.7	6 ±6.9
Condroz	Marchin $n=10$	12.7±12.6	6.5±16.5	52 ±79
	Perwez $n=7$	24.8±31	—	38.6±78
	St-Mort $n=6$	22.8±35.4	—	49.3±53.5

(—): below the detection level i.e. 0.1 ppb.
$n=$: number of samples.

1) The mean degree of contamination by lindane and DDT appeared higher in ants than in beetles.

2) As a consequence of high individual variations of insecticide contamination, there was no significant differences between the concentrations of residues measured on one hand in ants and on the other hand in beetles according to the sampling stations (agricultural and forested areas).

3) In spite of the legal interdiction of the utilization of the most organochlorine insecticides (with the exception of lindane) since 1975, residues were still detected in sapronecrophagous beetles and in ants. The contamination of insects was generally low although a few samples contained high concentrations of prohibited insecticides (up to 274 ppb of dieldrin and 419 ppb of DDT metabolites).

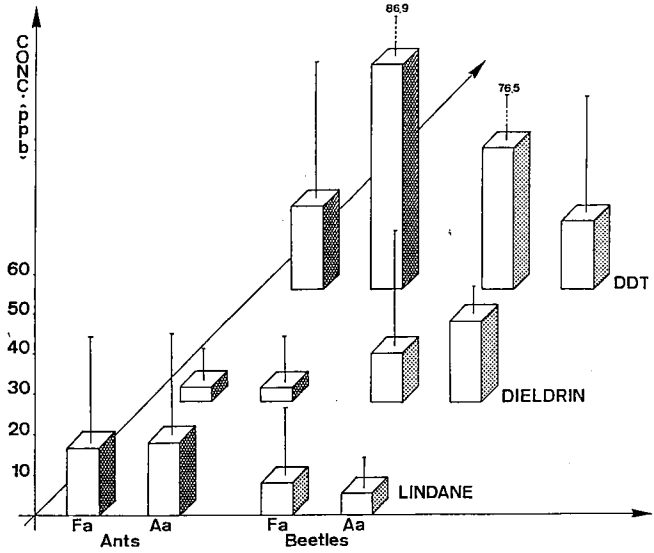

Figure 3 Total mean concentration (±standard deviation) of organochlorine insecticides in ants and in beetles in forested (Fa) and agricultural (Aa) areas.

The generalized contamination of insects by lindane could be explained by the use of lindane suspension or powder called GAMMA COL (I.C.I. no. 6210) in the cultivated areas and SYLVOGAM (I.C.I. no. 1721) in the forested one. However, the insect contamination by DDT and dieldrin in forested areas (where these insectides have never been used) would not result from a direct intoxication due to application of these compounds on the sampling sites. At the present time, it is well known that co-distillation phenomenon conjugated with atmospheric transport of contaminated particles would be a preferential way of entrance of xenobiotics in terrestrial environment.[10–11] As a consequence, these particles could be transported far away from the old and/or actual sites of DDT, dieldrin and lindane applications in the direction of the West dominant winds (in Belgium) to forested areas of Hautes Ardennes Orientales (Figure 1).

CONCLUSIONS

The low contamination of insects by prohibited organochlorine pesticides is undoubtedly a consequence of their legal interdiction in

most European countries and especially in Belgium. However, after 10 years, there is still a background level of contamination.

Nevertheless, such a contamination in insect populations is suspected to induce intoxication and metabolism troubles in insectivorous birds. Indeed, Stickel[12] suggested that regular intake of preys contaminated by organochlorine residues could induce up to 90 times biomagnification in insectivorous birds. Therefore, although final concentrations in insect predators would not be high enough to instigate acute lethal poisoning of predators, they are sufficient to elicit biological troubles such as steroid metabolism injury, birth defects, decrease in hatchability and in eggshell thickness, liver injury and troubles in microsomal mixed function oxydase mechanisms.[10–15] As a consequence, these troubles could cause a dramatic decrease of insectivorous bird populations which could even induce the disappearance of the species.

Then, ecotoxicologists have to be very careful before concluding in the favour of harmless effects that a low contamination of insects by remanent xenobiotics could induce to disturb the stability of terrestrial ecosystems.

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