Bull Environ Contam Toxicol (2011) 87:487–493
DOI 10.1007/s00128-011-0406-9

Sensitivity of Two *Mesocyclops* (Crustacea, Copepoda, Cyclopidae), from Tropical and Temperate Origins, to the Herbicides, Diuron and Paraquat, and the Insecticides, Temephos and Fenitrothion

C. Leboulanger · C. Schwartz · P. Somville · A. O. Diallo · M. Pagano

Received: 3 February 2011 / Accepted: 2 September 2011 / Published online: 11 September 2011

Abstract Ecotoxicological assessment in the tropics is based mainly on knowledge gained from temperate organisms, although many studies have shown the need for models that are more appropriate to tropical regions. The toxicity of two herbicides and two insecticides to nauplii of two freshwater zooplankton species, *Mesocyclops aspericornis* (von Daday in Inseln Zool Jb Syst 24:175–206, 1906) from a tropical reservoir and a *Mesocyclops* sp. from a temperate pond, were compared. Both strains were sensitive to paraquat, temephos and fenitrothion, tropical *M. aspericornis* being generally more tolerant (EC50s of 207, 1,450 and 1,840 μg L^{-1} for paraquat, temephos and fenitrothion, respectively) than the temperate *Mesocyclops* sp. (EC50s of 152, 45 and 1,017 μg L^{-1} for paraquat, temephos and fenitrothion, respectively) whereas diuron was only slightly toxic (LOEC = 446 μg L^{-1} for both strains). The nauplii mortality increased with the exposure time for both species during each experiment. Further isolation of model organisms from tropical ecosystems is needed, to meet the requirements for a tropical risk assessment scheme.

Keywords Zooplankton · Herbicide · Insecticide · Tropical and temperate model organisms

Experimental bioassays involving cultured and captive organisms are key steps in the environmental risk assessment of pesticides and chemicals, especially regarding aquatic ecosystems. The inadequacies of standard models, which are mainly organisms from temperate regions, are now well recognized for application to subtropical and tropical regions (Castillo et al. 1997; Kwok et al. 2007) and there is increasing concern in the context of ecotoxicology to determine new, indigenous species to be included in environmental risk assessment of chemicals (Moreira-Santos et al. 2005; Leboulanger et al. 2009; Menchaca et al. 2010). As relatively little is known about the chemical sensitivity of tropical species and environments, in comparison to the temperate counterparts that are often used as surrogates, further studies on the sensitivity of organisms from various origins are needed (Daam and Van den Brink 2010).

Freshwater cyclopoids are found in almost all types of freshwater habitat and *Mesocyclops aspericornis* has been reported in many water bodies in tropical and sub-tropical zones (Zehra and Altaff 2002). It can grow up to 1.5 mm and, as a predator in pelagic systems, has been proposed as a biological agent for mosquito larvae control (Kosiyachinda et al. 2003; Ramanibai and Kanniga 2008). Laboratory breeding of captive populations is easy, with a generation time close to 1 week (Kosiyachinda et al. 2003). Provided that *M. aspericornis* is not a complex of cryptic species, it could be a good candidate as a model zooplankton for freshwater risk assessment. These characteristics also support the proposal of alternate models of tropical daphniidae, such as for example *Ceriodaphnia*

C. Schwartz and P. Somville have contributed equally to this work.

C. Leboulanger (✉)
IRD, UMR5119 ECOSYM Université Montpellier II-CNRS-IRD-Ifremer-UM1, SMEL, 2 rue des Chantiers, 34200 Sète, France
e-mail: christophe.leboulanger@ird.fr

C. Schwartz · P. Somville · M. Pagano
IRD, UMR 213 LOPB, Centre d'Océanologie de Marseille, Case 901, 13288 Marseille Cedex 09, France

A. O. Diallo
ENSA, Université de Thiès, Km 9 route de Khombole, BP A296, Thiès, Senegal

species reared in the laboratory, to replace the temperate *Daphnia magna* in "tropical-designed" studies (Do Hong et al. 2004).

The two model herbicides tested were diuron and paraquat. Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a widely used photosystem II inhibitor, potentially affecting aquatic ecosystems throughout the world, even remote ones (e.g. Shaw et al. 2010). It is considered as being of low to moderate toxicity to tropical zooplankton (Leboulanger et al. in press) but significant chronic effects have been reported on pond zooplankton at levels down to 10 μg L^{-1} (Persbacher and Ludwig 2004; Zimba et al. 2002). Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) is a quaternary ammonium photosystem I inhibitor, with subsequent formation of reactive oxygen species in photosynthetic organisms. Despite regulatory measures and being banned in several countries, this herbicide is still widely used, especially in the tropics (e.g. Amondham et al. 2006; Sucahyo et al. 2008). Its toxicity to zooplankton is extremely variable, depending on the test conditions and target organisms (Leboulanger et al. 2009; Persbacher and Ludwig 2004).

The two model insecticides tested were fenitrothion (O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) and temephos ([4-(4-dimethoxyphosphinothioyloxyphenyl) sulfanylphenoxy]-dimethoxy-sulfanylidene-phosphorane), both organophosphorus inhibitors of nervous transmission in arthropods widely used mainly for mosquito control (Delatte et al. 2008). Only a few studies have investigated the toxic effects of temephos on zooplankton (Hanazato et al. 1989; Helgen et al. 1988) and Brown et al. (1999) reported that temephos was toxic to young stages of freshwater shrimps. However, the metabolic degradation of fenitrothion by zooplankton has been studied, illustrating the high tolerance level to this insecticide (Kashiwada et al. 1995, 1998). Recently, Kaur and Ansal (1996) and Lahr et al. (2000) demonstrated the toxicity of fenitrothion to zooplankton, and Choi et al. (2001) used respiratory electron transport systems as a biomarker of its toxicity to chironomid larvae.

Two lineages of cyclopid zooplankton belonging to the genus *Mesocyclops* were isolated, one from a temperate water body and one from a tropical water body, to test the toxicity of these four commonly used pesticides to these two closely related lineages. The nauplii stages were exposed to increasing concentrations of the pesticides for up to three days, to assess their acute sensitivity and avoid any indirect effect from exposure to contaminated diet.

Materials and Methods

Live tropical zooplankton were collected on March 2009 at the Combani Reservoir, Mayotte (12°46′39″S; 45°08′29″E) and temperate zooplankton were collected in July 2009 at Borély City Park, Marseilles (43°15′38″N; 5°22′57″E). Several multiresidue chemical analyses were carried out on the tropical island of Mayotte in 2007, 2008, and 2009 and no significant contamination of the Combani Reservoir by pesticides was found (Leboulanger et al. in press, and unpublished data). The contamination level of Borély pond is not known. The plankton were collected by vertical hauls using a 60 μm mesh plankton net, sorted immediately under binocular magnification and healthy *Mesocyclops* were taken from the whole community using plastic pipettes. The *Mesocyclops* were then kept in 5 L glass cylindrical aquariums, in de-chlorinated tap water (overnight air bubbling before each experiment) renewed every 2 weeks. Water was assumed to be free of significant contamination, according to the regulatory limits of .5 μg L^{-1} for the sum of pesticides (European Union 1998). Food was supplied weekly in the form of commercially available aquarium flakes (Tetra Min), and the aquariums were kept at 25°C in a climatic chamber with 12 h light–12 h dark cycle at 20 μmol photons m^{-2} s^{-1} for 1 year before the experiment.

Pure pesticides (purchased from Sigma-Aldrich: PEST-ANAL® analytical standards for fenitrothion, paraquat and temephos, and >98% purity for diuron) were weighed and dissolved in HPLC-grade methanol, stored at −20°C before use. Semi-logarithmic dilutions were prepared in methanol, and test solutions were prepared by diluting the stock solution in filtered tap water. The effective pesticide concentrations were determined using standard methods: diuron was measured using LC/DAD/MS and ^{2}H-simazine as internal standard, paraquat by LC/DAD and UV detection, whereas fenitrothion and temephos were analyzed using GC/MS with 1-bromo, 2-nitrobenzene as internal standard.

At the beginning of the experiment, newborn nauplii (<24 h old) were picked from the aquariums and 10–15 individuals were distributed in 40 mL plastic vials containing 20 mL of .45 μm filtered water. Quadruplicates were performed for each test concentration, together with a control with the same amount of methanol as test vessels (.05% v/v). Every 24 h for three successive days, dead *Mesocyclops* were counted and removed from the vessels.

Two-way ANOVA was performed to test, for each pesticide, the interaction between the strain and the response to the pesticide concentration. *Post hoc* comparisons, using the Scheffé test, were also performed to test the differences between the controls and the different concentrations for each pesticide and for each strain. All the analyses were performed using Statistica V.6 (StatSoft Inc., Tulsa, USA). When possible, EC50 was evaluated using the REGTOX macro for Excel® developed by E. Vindimian (http://www.normalesup.org/~vindimian/fr_index.html) estimating the upper and lower limits for parameters using Hill's model. This macro can be used to calculate EC50 and confidence intervals even in cases where not all individuals died at the highest concentrations tested.

Available data on the toxicity of diuron, paraquat, fenitrothion and temephos to zooplankton were retrieved from the US EPA ECOTOX database (http://cfpub.epa.gov/ecotox/) and only results regarding daphnia and copepods presumably from freshwater were kept for analysis for homogeneity. Species-sensitivity distribution curves (SSD) were drawn using the EC50 available for each pesticide.

Results and Discussion

No difficulties were experienced in rearing either *Mesocyclops* strain in the laboratory for several months. Both strains were sensitive to all pesticides in the concentration range tested, but with significant differences in the levels of sensitivity and time lag for the toxicity to become apparent (Table 1). In all tests, there was noticeable nauplii mortality in the controls after 48 and 72 h of incubation, revealing the sensitivity of the organisms to the test conditions during this early development stage (Fig. 1). This behavior is consistent with the development stages of cyclopoids, which rapidly require feeding for further molting and growth. In such a case, the duration of exposure should not exceed 48 h to ensure accurate routine evaluation of chemical toxicity, despite the evidence for late appearance of toxic effects for some compounds. A reliable estimation of pesticide toxicity can be made by monitoring the mortality with time, and when 48 h mortality was higher than 10% (this occurred once, with temephos as test molecule) the experiment was repeated.

The effective concentration of pesticides varied depending on the pesticide considered. Fenitrothion concentrations measured at the beginning of exposure were reduced to 49.1 ± 2.9% of the nominal concentration (n = 6). Fenitrothion has already been shown to be extremely labile in experimental conditions, with a further exponential decay during incubation at 25–28°C (Leboulanger et al. in press). Diuron concentrations varied around 86 and 96.5% of the nominal concentration, with a mean of

Table 1 Results of two-way ANOVA on concentration effects and exposure time for the two *Mesocyclops* strains

Pesticide		Concentration (µg L^{-1})	Borély (temperate)			Mayotte (tropical)		
			24 h	48 h	72 h	24 h	48 h	72 h
Paraquat		.5	1	.994	nd	1	1	nd
		4.9	1	.385	nd	1	.999	nd
		15.5	.995	.127	nd	1	.891	nd
	*	49	.999	**.000**	nd	1	.461	nd
	**	155	1	**.000**	**.000**	.880	**.000**	**.000**
	**	204	.179	**.000**	**.000**	1	**.000**	**.000**
	**	309	**.011**	**.000**	**.000**	**.000**	**.000**	**.000**
Diuron		3.4	1	.648	nd	1	1	nd
		10.7	1	.923	nd	1	1	nd
		34	.912	.843	nd	1	1	nd
		107	1	.944	nd	1	1	nd
		338	.935	.101	.848	1	.733	.922
	**	446	1	**.001**	**.002**	.925	**.002**	**.050**
	**	677	.995	**.001**	**.001**	.090	**.006**	.105
Fenitrothion		1.6	1	.982	nd	1	1	nd
		5.1	1	1	nd	1	1	nd
		16.2	1	.996	nd	1	1	nd
		51	1	.999	nd	1	.998	nd
		164	1	.119	.157	1	.096	.710
	*	213	.699	.683	**.036**	.058	.197	.613
	**	324	.519	.097	**.000**	.066	.107	**.026**
Temephos[a]		10.4	.788	.834	.323	1	1	1
	*	33	1	**.000**	**.000**	1	1	.989
	*	66	.137	**.000**	**.000**	1	.984	1
	*	330	**.010**	**.000**	**.000**	.964	.162	.996
	**	660	**.001**	**.000**	**.000**	.332	**.000**	.224

Post hoc comparison *p* values (Schéffé test) are indicated for each concentration compare to controls after 24, 48 and 72 h exposure. Significant effects ($p < .05$) highlighted in bold; concentrations toxic for one or two species are marked with * and **, respectively

[a] Temephos concentrations are nominal (see text)

91.5% (n = 6). Paraquat was reduced to 48% of the nominal concentrations (n = 6), as reported in a similar experiment (Leboulanger et al. in press). For fenitrothion, diuron and paraquat, the measured concentrations are used in the following section. Temephos was not found by the analytical procedure after samples had been stored for weeks at −20°C and so no effective concentration could be calculated. We chosen to work with plastic vessel rather than glassware, to ensure homogeneity of methods and results comparison when assessing the sensibility of wild zooplankton to selected pesticides (Leboulanger et al. 2009, and unpublished work) and evaluating the indirect effects on whole plankton communities in the field (Leboulanger et al. in press). Due to a low solubility and high log K_{ow} close to 6 (HSDB 2011), this compound would be rapidly adsorbed to the walls of the vessels. The results for temephos are presented using the nominal concentration instead of the measured concentration, taking in mind than actual concentrations would be lower and probably transient in the experimental systems.

The highest paraquat dose applied (309 µg L^{-1}) resulted in nauplii mortality after 1 day of exposure for both strains. For longer exposure, paraquat caused significant nauplii mortality at doses as low as 49 µg L^{-1} for temperate strains and 155 µg L^{-1} for tropical strains. Dose-response curves were drawn (example Fig. 2) and LOECs and EC50s were estimated from the ANOVA results (Table 1) and the REGTOX macro (Table 2), respectively. Paraquat EC50 for temperate *Mesocyclops* was lower than that for the tropical strain.

Diuron was only lightly toxic for both zooplankton species and the dose–response data set obtained was not relevant for EC50 estimation. Nevertheless, the two highest concentrations tested resulted in significant mortality of nauplii relative to the controls, with a LOEC of 446 µg L^{-1} for the two zooplankton strains.

Temephos was toxic for the temperate *Mesocyclops* after 1 day of exposure, at 330 µg L^{-1}, whereas the tropical strain remained unaffected for 2 days at the same concentration. As for paraquat, dose–response curves were analyzed and an estimation of EC50 was possible only for the temperate strain (45 µg L^{-1}, see Table 2). For the tropical strain, the calculated EC50 was between 960 and 3,044 µg L^{-1}, the uncertainty being due to the low and very variable mortality observed between the test replicates. Further, the actual temephos concentrations applied to the organisms have to be considered as significantly lower than the nominal ones, provided that the compound was undetectable after only few weeks of storage at −20°C.

Fenitrothion was almost non-toxic for both zooplankton strains, since mortality was significant relative to the controls only after 72 h of exposure, at 213 µg L^{-1} for the temperate organisms and 324 µg L^{-1} for the tropical organisms. The calculated EC50s for both strains were similar being close to a tenth of the reported water solubility for fenitrothion. The effects of fenitrothion on zooplankton observed in this study also reflected the results of other field experiments where a dose of 506 g a.i./ha fenitrothion, resulting in an average initial concentration of 80 µg L^{-1} within the water body treated, were used. Fenitrothion did not cause any significant reduction or increase in the average density of the cyclopids *Thermocyclops decipiens* and *Mesocyclops kieferi* or the calanoids *Tropodiaptomus banforanus* and *Paradiaptomus rex*. During the study period that took place in Senegal in 1992 the individual numbers of copepods in the treated ponds were slightly lower than in the control group, but not radically so (Lahr et al. 2000).

The species sensitivity distribution (SSD) curve obtained for the four pesticides (Fig. 3) showed different patterns depending on the compound. For paraquat, the lowest EC50s were close to .001 µg L^{-1}, but most of the species were affected at concentrations exceeding 1,000 µg L^{-1}. The EC50s values calculated from the present study for the two *Mesocyclops* strains lie in the lower 25% of the data set and it should be considered that this genus is more sensitive to paraquat than other taxons tested, mainly daphniidae. However, the two cyclopoid strains tested were tolerant to diuron (NOEC of 338 µg L^{-1}, Table 1) and their sensitivity to this herbicide should be considered as comparable to other species. Zimba et al. (2002) reported a decrease in zooplankton

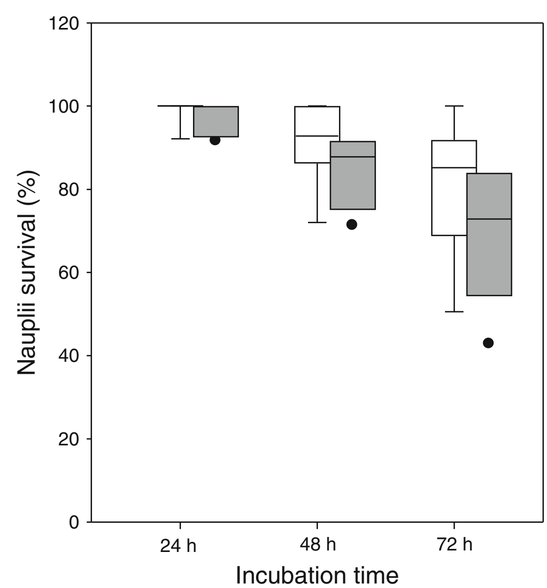

Fig. 1 Box and Whisker plot of cyclopid nauplii survival in control vessels during incubation. *Open bars*: *Mesocyclops aspericornis* from Mayotte; *shaded bars*: *Mesocyclops* sp. from Borély. Median and outlier values are shown

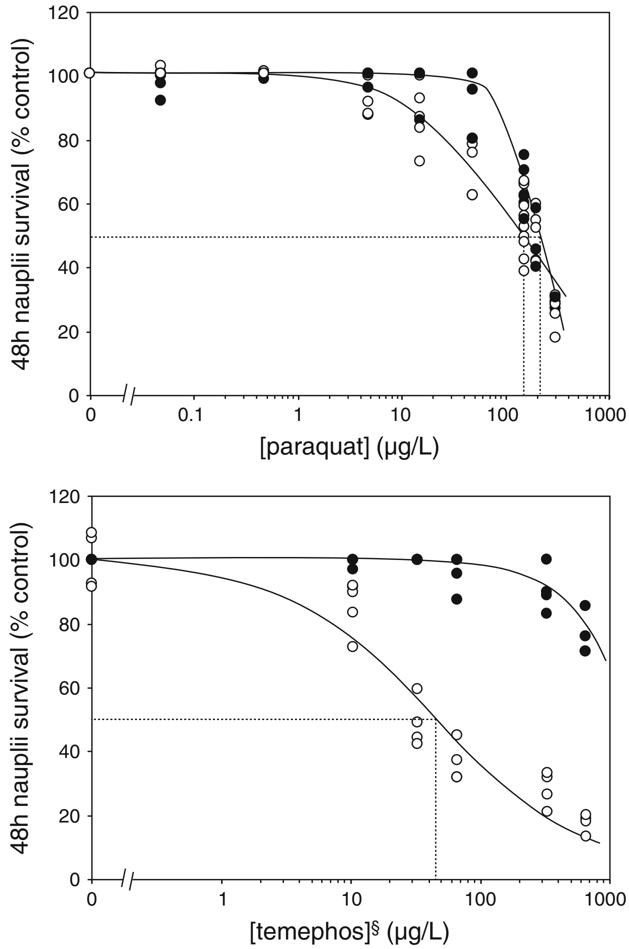

Fig. 2 Example of dose–response curves for temperate (*open symbols*) and tropical (*closed symbols*) *Mesocyclops* nauplii to paraquat (*upper panel*) and temephos (*lower panel*) exposure. Survival rate is estimated relative to controls after 48 h. *Section sign*: temephos concentration is the nominal concentration

density and a parallel increase in rotifer in ponds treated with 10 µg L^{-1} of diuron, whereas, in a previous study, no evidence was found of the direct toxicity of 11.4 µg L^{-1} of diuron on a zooplankton community from the same lake dominated by *Thermocyclops decipiens* in outdoor microcosms (Leboulanger et al. in press). These communities from the Combani reservoir also showed significant short-term toxic effects for paraquat at only 5.3 µg L^{-1}, whereas the two cladoceran species (*Moina micrura* and *Diaphanosoma excisum*) from Burkina Faso appeared to be more tolerant to paraquat with EC50 in the 50–60 µg L^{-1} range (Leboulanger et al. 2009). Both *Mesocyclops* strains used in this study were tolerant to fenitrothion compared to other zooplankton tested, as the EC50s were in the higher 5 percentile of the values reported. For temephos, the temperate strain was moderately sensitive, whereas the tropical strain was among the most tolerant zooplankton species, as shown by the SSD curve (Fig. 3).

In the light of these results, further studies are needed to construct a framework for environmental risk assessment of pesticides in tropical regions. This framework will benefit from field studies, which will help to select relevant organisms based on their known distribution throughout the tropical zone (Freitas and Rocha 2011; Do Hong et al. 2004; Sucahyo et al. 2008). With several notable exceptions, species from tropical ecosystems have so far been considered to be more tolerant to most of the chemicals tested compared with their temperate counterparts (Kwok et al. 2007; Daam and Van den Brink 2010). The underlying mechanisms explaining this difference still need to be elucidated, although it is possible that, where there is historical chemical exposure, there will be selection of tolerant zooplankton in the field (Brausch and Smith 2009). The search for the most sensitive species has been a topic for a long time (Cairns 1986; Cairns and Niederlehner 1987) but this is certainly not the primary task to be achieved. The success of some temperate zooplankton species such as the *Daphnia* ssp. for models in ecotoxicity testing is based on the distribution range of this group of species together with its ease of handling in the laboratory rather than because it is representative of natural plankton communities. Laboratory work is required to ensure the availability of tropical test organisms selected from the field by complete understanding of their life-cycle for the long term, before these models can be used for ecotoxicological assessments. Meanwhile, further studies are needed to ensure that the cultured organisms reflect the sensitivity of natural organisms in a measurable way, which has not always been the case (Menchaca et al. 2010, Freitas and Rocha 2011). Test protocols also need to be standardized, as factors such as

Table 2 Summary of EC50s for the two *Mesocyclops* strains calculated using REGTOX

Compound	Strain	EC50—48 h (µg L^{-1})	LOEC—48 h (µg L^{-1})
Paraquat	Tropical	207 (192–221)	155
	Temperate	152 (128–180)	49
Diuron	Tropical	>677	446
	Temperate	>677	446
Fenitrothion	Tropical	1,840 (613–7,511)	213
	Temperate	1,017 (661–2,224)	324
Temephos[a]	Tropical	1,450 (960–3,044)	660
	Temperate	45.0 (34.1–74.6)	33

Upper and lower confidence limits (95%) are given, LOEC are deduced from ANOVA

[a] Temephos concentrations are nominal (see text)

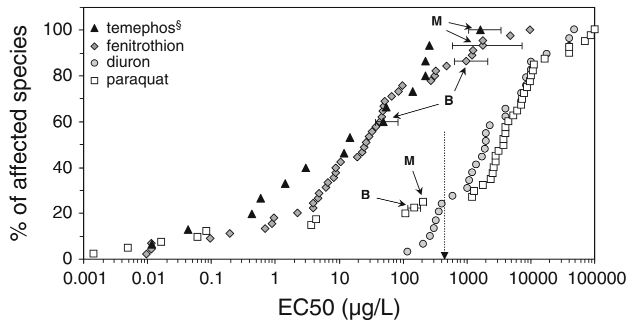

Fig. 3 Species-sensitivity distribution curve for temephos, fenitrothion, paraquat, and diuron toxicity for freshwater zooplankton, including data from this study with confidence intervals. M: Mayotte, tropical strain of *Mesocyclops aspericornis*; B: Borély, temperate strain of *Mesocyclops* sp. *Dashed arrow* indicates the LOEC of diuron for both strains. *Section sign*: temephos concentration is the nominal concentration

the surface/volume ratio of test vessels may affect the exposure conditions of organisms, especially in the case of toxic compounds with high sorption characteristics. Most of all, tropical freshwater ecosystems will only benefit by accelerated, efficient communication and international collaboration are required to achieve a consensus for the models and test procedures, similar to that in temperate countries for environmental risk assessment.

Acknowledgments This work was funded by the French Ministry for the Environment under the ECOMET project (grant n° CV70000783). We should like to thank Dr. Laurence Amalric, BRGM Orléans, France, for the pesticide analysis. Editor-in-Chief Dr. Herbert Nigg and anonymous reviewers are acknowledged for improvements on the earlier version of this manuscript. Tony Tebby edited English text.

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