Ecotoxicology and Environmental Safety **48,** 66–75 (2001)
Environmental Research, Section B
doi:10.1006/eesa.2000.1995, available online at http://www.idealibrary.com on IDEAL®

Acute Toxicity of Locust Insecticides to Two Indigenous Invertebrates from Sahelian Temporary Ponds

Joost Lahr,*[,1] Aliou Badji,† Simone Marquenie,‡ Egbertha Schuiling,‡ Khalifa B. Ndour,† Alpha O. Diallo,† and James W. Everts*

*Food and Agriculture Organization of the United Nations, Project GCP/SEN/041/NET (LOCUSTOX), B.P. 3300, Dakar, Senegal;
†Direction de la Protection des Végétaux, Projet LOCUSTOX, B.P. 20054, Thiaroye, Senegal; and
‡Van Hall Instituut, Postbus 1528, 8901 BV Leeuwarden, The Netherlands

Received February 19, 1999

During desert locust plagues large amounts of insecticides are used for control operations. Drift from these treatments and accidental overspraying may contaminate small surface waters such as temporary ponds. The present study describes methods for static acute toxicity tests with two abundant organisms that occur in temporary ponds in the African Sahel region: the fairy shrimp *Streptocephalus sudanicus* Daday (Branchiopoda, Anostraca, Streptocephalidae) and the backswimmer *Anisops sardeus* Herrich-Schåffer (Hemiptera, Notonectidae). The organisms were captured in the field and 48-h static toxicity tests were conducted in the laboratory. The assays were used to screen the toxicity of 11 formulated synthetic insecticides used in desert locust control and of spores of the mycopesticide *Metarhizium anisopliae* var. *acridum*. Most of the synthetic insecticides tested were highly toxic to both organisms (LC_{50} or $EC_{50} < 1$ mg/L). Exceptions were the toxicity of diflubenzuron to *A. sardeus* (moderately toxic: $1 < LC_{50} < 10$ mg/L), and that of fenitrothion (moderately toxic) and malathion (slightly toxic: $10 < EC_{50} < 100$ mg/L) to *S. sudanicus*. *M. anisopliae* var. *acridum* was moderately toxic to *S. sudanicus* and only slightly toxic to *A. sardeus*. EC_{50} values of the insecticides for *S. sudanicus* were not significantly correlated with $L(E)C_{50}$ values for *Daphnia magna* collected from the literature. For *A. sardeus* a significant correlation with *D. magna* was found, but even in this data set the two species had widely differing sensitivities to some insecticides.

Key Words: **temporary ponds; insecticides; aquatic invertebrates; toxicity testing; indigenous species; arid zones; desert locust control.**

INTRODUCTION

Plagues of desert locust, *Schistocerca gregaria* (Forskål), occur at irregular intervals in northern Africa and in parts of the Middle East and Asia. During such plagues the amounts of insecticides used against swarms and hopper bands are considerable (Everts, 1990). These operations, in particular when aircraft is used, may also contaminate small surface waters by accidental overspraying or by downwind drift. One example of such small aquatic habitats is temporary ponds, which occur seasonally in hot arid areas where rainfall is often low or concentrated in short periods. Several authors have addressed the importance of temporary ponds and the special hydrological and ecological features they possess (e.g., Cole, 1968; Belk and Cole, 1975; Williams, 1985).

Current aquatic risk assessment procedures for pesticides are based almost entirely on the toxicity of compounds to several European and North American standard species of algae, invertebrates, and fish. These may not necessarily be representative species for aquatic environments in other parts of the world. The need for the incorporation of data on indigenous species in risk assessment for tropical and hot arid and semiarid regions has been emphasized by Everts (1997), SETAC (1996), and Widianarko and Van Straalen (1997). Moreover, Lahr (1997) argued that the special adaptations that are found among organisms living in temporary ponds in these areas may affect the impact of toxicants on the individual, population, and community levels.

Results from comparative field experiments by Lahr *et al.* (2000) indicated that at the recommended dose rates, several locust insecticides may have a detrimental impact on the aquatic invertebrate fauna of temporary ponds in the Sahel. These studies also revealed groups of indicator species for the assessment of side effects on these ecosystems in the Sahel. These were fairy shrimp (Branchiopoda, Anostraca), backswimmers (Hemiptera, Notonectidae), and water fleas (Cladocera).

Depending on the availability of trial data on the efficacy against desert locust, the Pesticide Referee Group, which

[1] To whom correspondence may be addressed at Vrije Universiteit, Faculty of Biology, Department of Ecology and Ecotoxicology, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. E-mail: joost.lahr@wxs.nl.

0147-6513/01 $35.00

advises FAO, identifies suitable insecticides and efficient dose rates. The latest update (FAO, 1998) lists 11 compounds. Organophosphates, carbamates, and synthetic pyrethroids are the most commonly used against swarms of adult locust. Benzoyl urea compounds, which inhibit chitin synthesis, are effective against hopper bands. The mycopesticide *Metarhizium anisopliae* var. *acridum* (Deuceromycetes, Moniliales) is a recently developed biological control alternative to chemical locust insecticides.

The present study describes static acute toxicity tests for two characteristic pelagic macroinvertebrate species from temporary ponds in the Sahel: *Streptocephalus sudanicus* Daday (Branchiopoda, Anostraca, Streptocephalidae) and *Anisops sardeus* Herrich-Schåffer (Hemiptera, Notonectidae). Each constitutes a major part of the invertebrate biomass found in temporary ponds in the region. The aim of the experiments was to evaluate the feasibility of toxicity tests with each species, to establish the need for bioassays with these indigenous species through the comparison of results to those for the standard invertebrate test species, *Daphnia magna* Straus, and to screen formulated insecticides used for desert locust control for their hazard to invertebrate life in temporary ponds.

METHODS AND MATERIALS

Insecticides

The synthetic insecticides used in the study were all solvent-based liquid ultralow-volume (ULV) formulations. These are commonly used at application rates of 0.5 to 2.0 L/ha (FAO, 1998). Table 1 gives an overview of the tested compounds. In addition to the 11 compounds on the FAO list, including *M. anisopliae* var. *acridum*, tests were conducted with two candidate insecticides: a propoxur/phoxim mixture and the pyrethroid betacyfluthrin. *M. anisopliae* var. *acridum* spores are a green powder that contains approximately 5×10^{10} spores/g. For application in the field, the spores are dispersed in a mixture of 70% light paraffin oil (kerosene for example) and 30% vegetable oil. The fungus is produced from Strain No. IMI 330189, originally isolated from the grasshopper *Ornithacris turbida cavroisi* in Niger (LUBILOSA, 1994).

Test Procedures

Toxicity tests were conducted in the laboratory of the Senegalese Crop Protection Directorate (DPV) in Nioro du Rip in central Senegal (13°45 N, 15°46 W). The laboratory was supplied with groundwater from a depth of 40 m, which contained no added chlorine. The pH was neutral; conductivity was approximately 300 μS/cm. Because *A. sardeus* survived well in this water, it was used as the test medium for this species. *S. sudanicus*, on the contrary, suffered considerable mortality when transferred to it, most likely due to osmotic shock. For tests with *Streptocephalus*, therefore, water from the well was first demineralized by passing it through an ORC active carbon filter and two R_3 ion exchangers (Bioblock, France). Following demineralization, conductivity was adjusted to approximately 100 μS/cm by adding 0.47 g Griffin Instant Pond Powder (Fisons Ltd., UK) per liter. Survival of *S. sudanicus* in this reconstituted water was good. The pH varied slightly around 6. The

TABLE 1
Characteristics and Formulations of Tested Insecticides

Active ingredient	Chemical group	Commercial name	Conc. (g a.i./L)	Formulation	Manufacturer (current name)
Fenitrothion	Organophosphate	Sumithion	500	ULV	Sumitomo, Japan
Chlorpyrifos	Organophosphate	Dursban	225	ULV	Dow Elanco, UK
Malathion	Organophosphate	Fyfanon	1230	ULV	Cheminova, Denmark
Bendiocarb	Carbamate	Ficam	200	ULV	AgrEvo, UK
Propoxur + phoxim[a]	Carbamate + organophosphate	Volaton + Unden	42 + 258 = 300	ULV	Bayer, Germany
Deltamethrin	Pyrethroid	Decis	75	ULV	AgrEvo, France
Lambdacyhalothrin	Pyrethroid	Karate	40	ULV	Zeneca, Côte d'Ivoire
Betacyfluthrin[a]	Pyrethroid	Bulldock	12	ULV	Bayer, Germany
Diflubenzuron	Benzoyl urea	Dimilin	450	ODC[b]	Uniroyal Chemical,
			60	OF[c]	The Netherlands
Teflubenzuron	Benzoyl urea	Nomolt	50	ULV	Cyanamid, Germany
Triflumuron	Benzoyl urea	Alsystin	50	ULV	Bayer, Germany
Fipronil	Phenyl pyrazole	Regent	20	ULV	Rhône-Poulenc, France
Metarhizium anisopliae var. *acridum*	Mycopesticide	Green Muscle	5×10^{10} (spores/g)	None	IITA, Benin

[a] Candidate agents, not on the list of FAO (1998) for desert locust control.
[b] For *A. sardeus.*
[c] For *S. sudanicus.*

mineral composition of the test water for both species is provided in Table 2. The water was aerated at least 2 h before the tests were started.

Animals were collected from temporary ponds in the area around Nioro du Rip. Depending on the progress of the rainy season and the availability of the appropriate life stage, different ponds were used as a source. At arrival in the laboratory the animals were transferred to an aquarium and acclimated to test water for at least 2 h before the start of the tests. Because they were easily recognized, for both test species, only adult females were used.

The tests were conducted in 3-L glass beakers containing 2 L of test water each. Ten individuals were selected and transferred from the aquarium into each test vessel. For each test, two beakers, which only received a dose of the solvent used to dilute the insecticides, were used as controls. Between 5 and 10 beakers received logarithmically increasing concentrations of the compounds. Each test was repeated at least three times with slightly different concentrations.

Dilution series of synthetic insecticides were prepared in analytical-grade acetone (min. 99.5% pure). The desired concentrations in the test vessels were obtained by adding between 100 and 1000 µL of the appropriate insecticide solution to the beakers using adjustable micropipets. Prior to this addition, each test vessel received a quantity of pure acetone equal to 1000 µL minus the volume of the insecticide solution to be added. Controls each received 1000 µL acetone. Following dosing, the water in the test vessels was stirred gently using a stainless-steel baton with a Teflon knob.

In tests with *M. anisopliae* var. *acridum*, no formulation was used because the aim was only to assess whether the fungus itself would cause an effect. An initial suspension of the highly hydrophobic spores was prepared by adding 1.6 g to 2 L test water. Ten drops of a liquid kitchen detergent was then added. The mixture was thoroughly homogenized on a magnetic stirrer for 2 h. This suspension was added to the test beakers in different quantities. A similar amount of detergent was added to the controls. Because *M. anisopliae* var. *acridum* is a slowly acting agent, the bioassays were continued for 96 h.

TABLE 2
Mineral Composition and Chemical Oxygen Demand (COD) of Water Used for Tests with *Streptocephalus sudanicus* and *Anisops sardeus*[a]

Parameter	Concentration (mg/L)	
	Reconstituted water (*S. sudanicus*)	Groundwater (*A. sardeus*)
Cl^-	23.7	6.5
SO_4^{2-}	< 0.05	30.9
NO_3^-	< 0.05	1.5
F^-	< 0.02	0.2
HPO_4^{2-}	< 0.05	0.9
HCO_3^-	2.4	105
NH_4^+	< 0.05	< 0.05
K^+	5.2	0.7
Ca^{2+}	0.3	32.5
Na^+	< 0.05	4.5
Mg^{2+}	7	9.1
Hardness	29.6	118.6
COD	98	45

[a] Variables were measured using ionic capillary electrophoresis by the ORSTOM Laboratory in Dakar, Senegal. Hardness of Ca^{2+} and Mg^{2+} is expressed in $CaCO_3$ equivalents.

Observations during Testing

After dosing, the beakers were placed on a table in front of the laboratory window, but out of direct sunlight. Thus, a natural light regime was maintained during the tests. In Senegal the photoperiod is more or less constant throughout the year. Day and night last about 13 and 11 h, respectively. During the tests, a constant temperature of ca. 27°C was maintained in the laboratory. Effects in each beaker were assessed after 24 and 48 h. The response of *A. sardeus* was straightforward. They were either alive or dead. The toxicity parameters used for this species were the 24- and 48-h LC_{50}. *S. sudanicus* displayed a more complex range of reactions after exposure to insecticides. The principal effect prior to death was immobilization. Immobilized individuals still moved their thoracic appendages, but were hardly able to swim. An animal was called "immobile" when it would remain on the bottom of the beakers for 10 s or more of observation. Immobilized animals never recovered. Another response of *S. sudanicus* was observed only after exposure to pyrethroids. *S. sudanicus* would frantically swim in circles at the water surface, apparently unable to submerge and orientate itself. These animals were registered in a separate, "seriously disoriented" category. On very few occasions individuals recovered from disorientation, but in general, individuals affected by pyrethroids first became disoriented, and later became immobile and died. The toxicity parameters for *S. sudanicus* are the 24- and 48-h EC_{50} (immobilization + disorientation + death).

Physical and chemical parameters were measured in the controls. Temperature and dissolved oxygen (DO) were determined at the start of each test (0 h) and after 24 and 48 h. Conductivity and pH are reported only for 0 h.

To check for possible external growth of the fungus, on several occasions, dead organisms in tests with *M. anisopliae* var. *acridum* were carefully removed from the beakers and incubated at room temperature. They were placed on moist filter papers in Petri dishes, sealed with a piece of Parafilm to prevent desiccation, and kept in the laboratory at ambient temperatures. After 20 h (the incubation time of

M. anisopliae var. *acridum*), the organisms were examined under a microscope (64 ×).

Statistical Analysis and Test Criteria

EC_{50} and LC_{50} values were calculated with the parametric method of Kooijman (1981) using the computer program from the Institute for Inland Water Management and Waste Water Treatment (RIZA) in The Netherlands.

Several test criteria recommended by the ASTM (1989) were applied. Results were accepted only when survival in the controls after 48 h was 90% or more. However, a control mortality of 15% was accepted for the 96-h assays with *M. anisopliae* var. *acridum*. Another criterion applied was that test series needed to include at least one concentration at which the proportion of affected organisms was 37% or less and one concentration that had an effect on 63% or more of the animals. An additional criterion to the ASTM guidelines concerned the width of the 95% confidence interval of the EC_{50} or LC_{50} values. When the lower limit was less than $0.5 \times L(E)C_{50}$ or when the upper limit exceeded $2 \times L(E)C_{50}$ the results were not judged valid, and the test was repeated. Each insecticide was tested three to six times for each species. Eventually the toxicity of an agent was expressed as the geometric mean of the three to six separate EC_{50} or LC_{50} values.

Sensitivities of the indigenous species were compared with that of *D. magna* by plotting the decimal logarithms of the LC_{50} or EC_{50} values for the insecticides, expressed as micromoles per liter, against the same data for the cladoceran. *D. magna* data were collected from open literature or obtained from the Dutch pesticide registration facility CTB. Least-squares linear regression with these data pairs was performed using a subroutine of Corel's QuattroPro program (Version 7.0) for Windows 95. Correlation coefficients were tested for significance using a one-sided *t* test according to Steel and Torrie (1980).

RESULTS

Test Performance

Water temperatures during the tests were fairly constant. The average for all tests was ~27.0°C In comparison, maximum daily water temperatures measured in natural temporary ponds in the Nioro du Rip area usually fluctuated around 30°C (Lahr *et al*., 2000). The average pH values of the water at the beginning of the tests were 5.9 ± 0.4 and 6.9 ± 0.5 (mean ± SE) for *S. sudanicus* and *A. sardeus*, respectively. The range between these two values more or less represents the variation that is found in the field (Lahr *et al*., 2000). The average conductivity at the beginning of the tests was 99 ± 5 µS/cm (mean ± SE) in the water used for *S. sudanicus* and 296 ± 10 µg/L for *A. sardeus*. Initial DO content was between 80 and 100% for most of the tests. In assays with *A. sardeus* this percentage dropped to an average of $36 \pm 24\%$ (mean ± SE) after 48 h. This large drop may be explained by the fact that the untreated well water may have contained bacteria and some dissolved organic matter. However, because *A. sardeus* is a surface breather it will not likely suffer from low oxygen levels in the water. The levels in the tests with *S. sudanicus* after 48 h dropped only to $56 \pm 11\%$ on average.

Mortality in the controls was usually low. Average control mortalities after 24 and 48 h were, respectively, 0.9 ± 2.2 (mean ± SE) and $2.3 \pm 3.6\%$ for *S. sudanicus*, and 0.5 ± 1.3 and $0.8 \pm 1.8\%$ for *A. sardeus*.

Toxicity

The EC_{50} and LC_{50} values calculated from the bioassays with the synthetic insecticides are summarized in Table 3.

When the oil flowable (OF) formulation of diflubenzuron (in diesel) was tested with *A. sardeus*, mortality at higher insecticide concentrations was extremely rapid (in a matter of minutes). It was noted that at high concentrations the formulation formed a microlayer on the water surface of the beakers and that individuals died shortly after they came to the surface to breathe. Therefore it seems plausible that clogging of their respiratory tubes by diesel caused the observed effect, i.e., not the molt-inhibiting active ingredient. Diflubenzuron tests with *A. sardeus* were therefore continued using the oil-dispersable concentrate (ODC) formulation instead, which contains no diesel. The effects of this formulation occurred much less instantly and at concentrations at least 100 times higher than for the OF formulation. The formulations of the other insect growth regulators (IGRs), teflubenzuron and triflumuron, which did not contain diesel, provoked a rapid lethal response as well, although somewhat less instantly. It is assumed that the mechanism of these effects was similar to that of the OF formulation of diflubenzuron.

The results for *M. anisopliae* var. *acridum* are given in Table 4. Replicate tests were highly variable during the first 48 h, but after 72 h, $L(E)C_{50}$ values started to converge. Many *S. sudanicus* that died in the tests displayed green spots or were completely covered by a greenish film. However, these symptoms did not much resemble the characteristic external sporulation observed in terrestrial arthropods that die due to exposure to the fungus (Danfa and van der Valk, 1999). In some cases the green spots would increase in size during incubation, but it could not be confirmed whether the observations represented genuine growth of the fungus. Neither was there a clear dose–effect relationship between occurrence of spots and films and spore concentrations (Fig. 1). In some of the animals that did not display green spots or films after death, these appeared during incubation. None of the *S. sudanicus* that perished in control tests displayed spots or films, neither before nor after

TABLE 3
Results of 48-h Acute Toxicity Tests with Females of *Anisops sardeus* (Hemiptera, Notonectidae) and *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and Different Formulated Insecticides Used to Control Desert Locusts[a]

	Streptocephalus sudanicus EC_{50} (µg/L)		*Anisops sardeus* LC_{50} (µg/L)	
Insecticide	24 h	48 h	24 h	48 h
Fenitrothion	3468 (3376–3657) $n = 3$	1230 (1102–1309) $n = 3$	16.7 (14.9–20.8) $n = 3$	8.61 (7.8–9.3) $n = 3$
Chlorpyrifos	8.25 (6.19–9.54) $n = 3$	3.48 (3.05–4.55) $n = 3$	1.58 (1.53–1.62) $n = 3$	0.90 (0.88–0.92) $n = 3$
Malathion	> 145,000[b] $n = 3$	67,750 (52,220–90,300) $n = 3$	70.7 (57.4–78.0) $n = 3$	42.2 (40.5–44.9) $n = 3$
Bendiocarb	71.5 (55.6–95.3) $n = 3$	41.0 (31.0–54.7) $n = 3$	389 (287–567) $n = 3$	373 (275–567) $n = 3$
Propoxur + phoxim	2.88 (1.65–4.42) $n = 6$	1.19 (0.90–1.68) $n = 6$	3.38 (2.65–4.52) $n = 4$	1.91 (1.75–2.08) $n = 4$
Deltamethrin	0.035 (0.026–0.051) $n = 3$	0.018 (0.017–0.019) $n = 3$	0.013 (0.011–0.014) $n = 3$	0.012 (0.010–0.014) $n = 3$
Lambdacyhalothrin	0.183 (0.181–0.186) $n = 2$	0.028 (0.024–0.032) $n = 2$	0.026 (0.024–0.031) $n = 3$	0.025 (0.023–0.031) $n = 3$
Betacyfluthrin	0.010 (0.006–0.021) $n = 4$	0.004 (0.003–0.006) $n = 4$	0.021 (0.016–0.026) $n = 4$	0.019 (0.015–0.025) $n = 4$
Diflubenzuron	13.3[c] (12.8–14.0) $n = 3$	0.74[c] (0.60–0.88) $n = 3$	2123[b,d] (1960–2210) $n = 3$	1937[b,d] (1800–2020) $n = 3$
Teflubenzuron	23.6[b] (9.47–43.40) $n = 5$	0.59 (0.24–1.66) $n = 5$	249[b] (233–267) $n = 3$	249[b] (233–267) $n = 3$
Triflumuron	17.5 (6.15–31.3) $n = 3$	0.21 (0.14–0.26) $n = 3$	199[b] (168–250) $n = 4$	189[b] (168–228) $n = 4$
Fipronil	65.4 (47.0–83.3) $n = 3$	9.94 (9.53–10.21) $n = 3$	20.2 (16.3–26.6) $n = 3$	9.06 (7.55–12.03) $n = 3$

[a] Data are presented as the geometric mean EC_{50} or LC_{50} and the table includes the range of values observed (minimum–maximum EC_{50} or LC_{50}, in parentheses) and the number of repeated tests n conducted with each compound and each test species, on which the mean was based. Concentrations refer to the active ingredient only.
[b] Higher than solubility in water according to Tomlin (1994).
[c] OF formulation.
[d] ODC formulation.

incubation, and none of the incubated specimens of dead *A. sardeus* developed growth of the fungus. Green spots and films were also absent in this species at all concentrations.

The 48-h L(E)C_{50} values from Table 3 were compared with the toxicity classification scheme of Canton *et al.* (1991). Most insecticides were highly toxic to *S. sudanicus* and *A. sardeus* (EC_{50} or $LC_{50} < 1$ mg/L), the three pyrethroids being the most toxic compounds to both species. The three benzoyl urea compounds were also highly toxic to *S. sudanicus*, but much less to *A. sardeus*. The toxicity of organophosphate insecticides to *S. sudanicus* varied considerably. Chlorpyrifos, for instance, was approximately

TABLE 4
Results of 96-h Acute Toxicity Tests with Females of *Anisops sardeus* (Hemiptera, Notonectidae) and *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and the Mycopesticide *M. anisopliae* var. *acridum*[a]

	24 h	48 h	72 h	96 h
S. sudanicus EC_{50} (mg/L)	> 6.8	7.1 (4.8–9.4)	4.4 (3.7–4.8)	3.0 (2.9–3.2)
	$n = 3$	$n = 3$	$n = 3$	$n = 3$
A. sardeus LC_{50} (mg/L)	> 20.7	23.8 (6.1–98.4)	18.2 (6.0–49.6)	11.4 (4.2–30.3)
	$n = 4$	$n = 4$	$n = 4$	$n = 4$

[a] Data are presented as geometric mean EC_{50} or LC_{50} value and the table includes the range of values observed (minimum–maximum EC_{50} or LC_{50}, in parentheses) and the number of repeated tests n conducted with each test species, on which the mean was based.

TABLE 5
Acute Toxicity of Insecticides Used in Desert Locust Control to *Daphnia magna* (Branchiopoda, Cladocera)[a]

Insecticide	Geometric mean L(E)C_{50} (µg/L)	References
Fenitrothion	11	Sanders *et al.* (1983), LeBlanc (1984)
Chlorpyrifos	1.3	Barron and Woodburn (1995), Kerstring and Van Wijngaarden (1992)
Malathion	1	Van Rijn *et al.* (1995)
Bendiocarb	74	Visser and Linders (1992), Tomlin (1994)
Deltamethrin	0.8	Van Rijn *et al.* (1995)
Lambdacyhalothrin	0.35	Hill (1989), Mokry and Hoagland (1990), Van Rijn *et al.* (1995)
Betacyfluthrin	0.72	Tomlin (1994)
Diflubenzuron	8.0	Hansen and Garton (1982), Mayer and Ellersieck (1986)
Teflubenzuron	0.47	Cyanamid Agro b.v. (J. Anthonissen, personal communication), CTB (1999)
Triflumuron	225	Tomlin (1994)
Fipronil	190	Tomlin (1994)

[a] Data represent the geometric mean LC_{50} and/or EC_{50} values from static tests. Most tests were performed with technical-grade insecticides, but a flowable formulation or a wettable powder was used on a few occasions.

20,000 times more toxic to *S. sudanicus* than malathion to which the fairy shrimp was surprisingly tolerant. If the same classification is also applied to *M. anisopliae* var. *acridum* (96-h data), the fungus may be considered moderately toxic to *S. sudanicus* ($1 < EC_{50} < 10$ mg/L) and only slightly toxic to *A. sardeus* ($10 < LC_{50} < 100$ mg/L).

Comparison to Daphnia magna

Collected acute toxicity data for *D. magna* are summarized in Table 5. No *Daphnia* data were obtained for the propoxur/phoxim mixture and for *M. anisopliae* var. *acridum*. Figure 2 presents the relative toxicity of the 11 remaining compounds to *S. sudanicus* and *A. sardeus* compared with that to *D. magna*. Linear regression revealed that the overall acute toxicity of the compounds to *A. sardeus* and to *D. magna* was significantly and positively correlated ($r^2 = 0.32$, $P < 0.05$). The relation is described by

$$\log y = 0.99 \times \log x + 0.10,$$

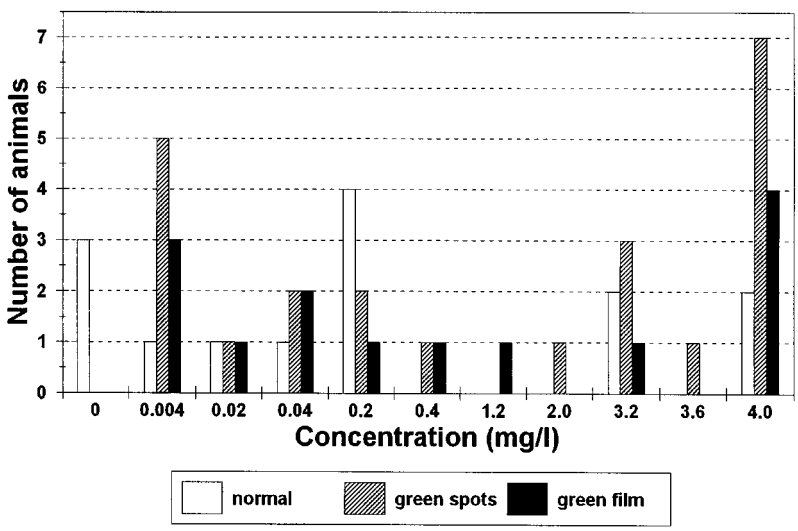

FIG. 1. Results of microscopic investigation after 20 h incubation of *Streptocephalus sudanicus* (Branchiopoda, Anostraca) that perished in toxicity tests with the fungus *Metarhizium anisopliae* var. *acridum*. The figure combines the observations from separate tests.

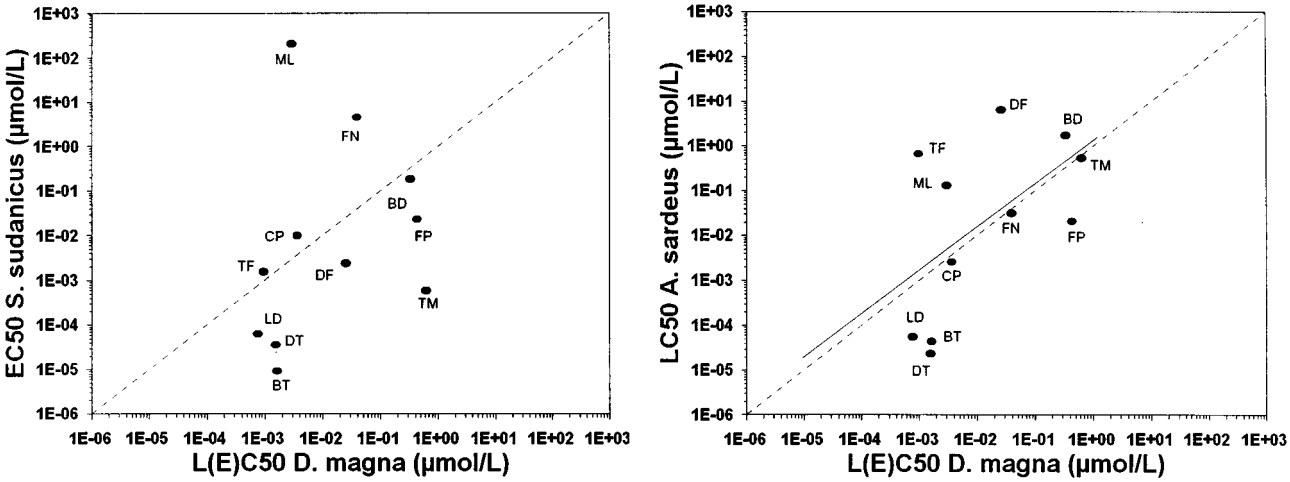

FIG. 2. Toxicity of insecticides used for the control of desert locust to *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and *Anisops sardeus* (Hemiptera, Notonectidae) compared with *Daphnia magna* (Branchiopoda, Cladocera). Dotted lines indicate equal toxicity to two species. The solid line for *A. sardeus* indicates the result of linear regression with significant correlation. BD, bendiocarb; BT, betacyfluthrin; CP, chlorpyrifos; DF, diflubenzuron; DT, deltamethrin; FN, fenitrothion; FP, fipronil; LD, lambdacyhalothrin; ML, malathion; TF, teflubenzuron; TM, triflumuron.

where y is the LC_{50} for *A. sardeus* and x is the $L(E)C_{50}$ for *D. magna*. The regression line that resulted from this equation (Fig. 2) approaches the line of equal toxicity of the substances to both species. Correlation between the data for *S. sudanicus* and *D. magna* was positive but not significant (regression coefficient = 0.57, $r^2 = 0.08$, $P > 0.05$).

DISCUSSION

Methods

The ASTM criteria concerning control mortality and percentage kill at the lowest and highest test concentrations were on most occasions easily met with both tests. However, compliance with other existing criteria may still be improved. Among others, organisms were not acclimated to test water for 48 h prior to each test, as the ASTM demands, but only for 2 h. This relatively short period improved survival in the controls after 48 h because the organisms, notably *S. sudanicus*, often became less fit after more than 96 h without feeding. Chemical analysis of concentrations in the test vessels could also be a useful complement to these static tests. In the present study, $L(E)C_{50}$ values were based solely on nominal initial concentrations. Some removal of insecticides will undoubtedly occur. For the current purpose however, which was to develop tests that can be used for risk assessment of single contamination events, static tests are an appropriate tool. Moreover, analysis of the active ingredient is not explicitly demanded by the ASTM or the OECD (1984) and, in addition, a theoretical framework for dealing with degradation and toxicokinetics in static tests has recently been proposed by Widianarko and Van Straalen (1996).

The development of more standardized tests with both species, using individuals cultured in the laboratory, may be considered. Rearing and feeding methods for several species of *Streptocephalus* have been described, although not for *S. sudanicus* itself (e.g., Moore, 1957; Sam, 1979; Brendonck *et al.*, 1990; Coutteau *et al.*, 1992; Maeda-Martínez *et al.*, 1995). Because fairy shrimp produce drought-resistant cysts, they have recently received increased attention for use in cost-effective cyst-based toxicity tests (Brendonck and Persoone, 1993). A test with one *Streptocephalus* species from southeast Africa, *S. proboscideus*, has been developed (e.g., Centeno *et al.*, 1992, 1993a, b), and tests have also been carried out with *S. rubricaudatus* and *S. texanus*, two species from northern Africa and North America, respectively (Crisinel *et al.*, 1994). A cyst-based version of the *S. proboscideus* test has been commercially available for some time as Streptotoxkit F (Persoone, 1998). This test, however, has recently been replaced by Thamnotoxkit F with the fairy shrimp *Thamnocephalus platyurus*, a species from the southwestern United States (Persoone, 1998; Centeno *et al.*, 1995).

To present knowledge, there has never been an attempt to culture *Anisops* species in the laboratory. The development of successful culturing methods for the species, as with most aquatic insects that have a terrestrial or flying dispersal phase during their life cycle, will be rather complicated. However, tests with organisms from natural temporary ponds produced very straightforward results and are easy to carry out.

Toxicity

A. sardeus and *S. sudanicus* were highly sensitive to most of the insecticides screened in this study. The lower toxicity

of benzoyl urea compounds to *A. sardeus* can be explained by the fact that adult stages of insects are usually much less sensitive to chitin synthesis inhibitors. *S. sudanicus*, on the contrary, molts continuously during its life cycle and is therefore more sensitive to these IGRs.

LC_{50} and EC_{50} values for the three benzoyl urea compounds sometimes exceeded the solubility in pure water (solubilities given by Tomlin, 1994), notably in the tests with *A. sardeus*. However, distinct dose–effect relationships were nonetheless obtained on all these occasions. At these instances, it is therefore preferred to report the calculated $L(E)C_{50}$ values instead of the solubilities (80, 19, and 25 μg/L for diflubenzuron, teflubenzuron, and triflumuron, respectively). This is allowed by the guidelines of the ASTM (1989). However, as pointed out before, the effects of these three insecticides on *A. sardeus* may also have been provoked by the formulations in addition to the active ingredients. This would explain why mortality occurred relatively quickly; i.e., 24- and 48-h LC_{50} values were more or less similar.

An unexpected result of this study was the large difference between the toxicities to *S. sudanicus* of the three organophosphates tested (Table 3). The 24-h EC_{50} for malathion and *S. sudanicus* was higher than its solubility (145 mg/L) and the mean 48-h EC_{50} was 68 mg/L. Fourty-eight-hour $L(E)C_{50}$ values for *A. sardeus* and *D. magna* were 42.2 and 1 μg/L, respectively. The low toxicity of malathion to *Streptocephalus* in the present experiments confirms the results of Calleja *et al.* (1994) and Crisinel *et al.* (1994) who reported 24-h LC_{50} values of the substance for three *Streptocephalus* species ranging from 54.6 to 81.5 mg/L, even though these investigators used larvae instead of adults. Because malathion (a phosphorodithioate with a succinate leaving group) belongs to another group of organophosphates than chlorpyrifos and fenitrothion (both phosphorothioates, but with a heterocyclic and a phenyl leaving group, respectively), these results indicate that there may exist a biochemical basis for the relative insensitivity of *Streptocephalus* to malathion. The succinate moiety may, for instance, be more easily transformed by deethylation as in many noninsects.

Although a clear dose–response relationship was found between the test suspensions of the spores of *M. anisopliae* var. *acridum* and the effects on both species, it could not be substantiated that these effects were caused by growth of *M. anisopliae* var. *acridum* into the organisms. The green spots and films observed did not have the appearance of ordinary *Metarhizium* growth. A possible explanation for the toxicity of the mycopesticide is secondary, toxic by-products. Such destruxins (DTXs) can be produced and excreted during growth of a mycelium into organisms (Charnley, 1992), possibly before actual sporulation takes place. In this way fungi may cause lethal effects while no external signs of sporulation are visible. But the action of *M. anisopliae* var. *acridum* on *S. sudanicus* may also have been of a mechanical nature. In tests with high concentrations of spores it was observed that the thoracic appendages of the animals, which are used for feeding and swimming, became green with spores and might have been clogged. The mechanism of the effect of *M. anisopliae* var. *acridum* on *A. sardeus* remains unknown as well.

The test exposure of the two aquatic species to *M. anisopliae* var. *acridum* represented a worst-case scenario because a synthetic detergent was used. Given the considerable effort that was needed to disperse the spores, even after addition of the detergent, it is unlikely that operational applications with the fungus would result in equally high concentrations of spores in surface waters.

Comparison to Daphnia magna

Crisinel *et al.* (1994) found that the sensitivity of three *Streptocephalus* species to four heavy metals was slightly higher than that of *Daphnia magna*. For 12 organic and organometallic compounds, toxicity was of the same order of magnitude. In another study, Persoone *et al.* (1994) evaluated the sensitivity of *S. proboscideus* (Streptotoxkit F) and *T. platyurus* (Thamnotoxkit F) larvae to a large number of pure toxic substances and environmental samples and compared it with that of *D. magna* using regression analysis. They found a significant positive correlation between the two species. Current results for 11 formulated insecticides and using adult organisms, on the contrary, indicated that standard tests with *Daphnia* may have little or no relation to the toxicity of these compounds to *S. sudanicus*, despite the fact that both species belong to the crustacean order of Branchiopoda. Compared with *D. magna*, *S. sudanicus* was more sensitive to synthetic pyrethroids such as lambda-cyhalothrin and betacyfluthrin, but less sensitive to organophosphates such as fenitrothion and malathion. A possible additional cause for these differences is that the actual insecticide concentrations in these tests may have been different from the nominal concentrations; i.e., they were not measured while some of the concentrations for the reported *D. magna* tests were. However, neither this difference in test design nor the difference between formulated and unformulated active ingredients can possibly account for the magnitude of the differences in toxicity observed for some compounds.

Results for *A. sardeus* were in closer agreement with standard *D. magna* assays with the same active ingredients. However, the difference observed was on some occasions still considerable, e.g., up to 670-fold for teflubenzuron. Hence, if safe concentrations of desert locust insecticides for temporary ponds in the Sahel were to be deduced, the use of *D. magna* as the sole species to represent the invertebrate fauna would constitute a large and unacceptable uncertainty.

CONCLUSIONS

In this study it was demonstrated that static acute toxicity tests with two aquatic invertebrates from the African Sahel region, *Anisops sardeus* and *Streptocephalus sudanicus*, which were captured in the field, gave satisfying, repeatable results and were relatively easy to carry out. A screening exercise with 11 synthetic insecticides and one mycopesticide formulated for desert locust control revealed that most synthetic compounds were highly toxic to both species. Exceptions were a lower toxicity of benzoyl urea compounds to *A. sardeus*, a highly variable toxicity of different organophosphates to *S. sudanicus*, and a relatively low toxicity of the mycopesticide *Metarhizium anisopliae* var. *acridum*. It is concluded that toxicity tests with *A. sardeus* and *S. sudanicus* yield important data to evaluate the potential ecological effects of insecticides in temporary ponds in the Sahel that cannot be accurately predicted by standard *Daphnia* tests alone. These results therefore support the use of relevant indigenous test species in local and regional risk assessment.

ACKNOWLEDGMENTS

The authors were supported by LOCUSTOX, a joint program of the Food and Agriculture Organization of the United Nations and the Government of Senegal. Its principal donor is the Government of The Netherlands. The views expressed in this paper represent those of the authors, and not necessarily those of the Food and Agriculture Organization of the United Nations. They thank Vincent Diémé and various other LOCUSTOX personnel for their contributions to the work. They are also grateful to Kees van de Guchte of RIZA in The Netherlands, for providing a copy of their LC_{50} computer program. All pesticide manufacturers mentioned kindly provided test samples of their products free of charge. Many thanks to Nico van Straalen, Vrije Universiteit, Amsterdam, for his critical evaluation of the manuscript and to Theo Brock and René van Wijngaarden, Alterra, Wageningen, The Netherlands, for their help with finding additional literature.

REFERENCES

American Society for Testing and Materials (ASTM) (1989). *Standard Guide for Conducting Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*, Designation: E729-88a. ASTM, Philadelphia.

Barron, M. G., and Woodburn, K. B. (1995). Ecotoxicology of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* **143,** 1–93.

Belk, D., and Cole, G. A. (1975). Adaptational biology of desert temporary-pond inhabitants. In *Environmental Physiology of Desert Organisms* (N. F. Hadley, Ed.), pp. 207–226. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Brendonck, L., and Persoone, G. (1993). Biological/ecological characteristics of large freshwater branchiopods from endorheic regions and consequences for their use in cyst-based toxicity tests. In *Progress in Standardization of Aquatic Toxicity Tests* (A. M. V. M. Soares and P. Calow, Eds.), SETAC Special Publications Series, pp. 7–35. Lewis, Boca Raton, FL.

Brendonck, L., Uyttersprot, G., and Persoone, G. (1990). A culture system for fairy shrimps (Crustacea, Anostraca). *Aquacult. Eng.* **9,** 267–283.

Calleja, M. C., Persoone, G., and Geladi, P. (1994). Comparative acute toxicity of the first 50 MEIC chemicals to aquatic non-vertebrates. *Arch. Environ. Contam. Toxicol.* **26,** 69–78.

Canton, J. H., Linders, J. B. H. J., Luttik, R., Mensink, B. J. W. G., Panman, E., Van de Plassche, E. J., Sparenburg, P. M., and Tuinstra, J. (1991). *Catch-up Operation on Old Pesticides: An Integration*, Rep. No. 678801002. RIVM, Bilthoven.

Centeno, M. D., Brendonck, L., and Persoone, G. (1992). Cyst-based toxicity tests. III. Development and standardization of an acute toxicity test with the freshwater anostracan crustacean *Streptocephalus proboscideus*. In *Progress in Standardization of Aquatic Toxicity Tests* (A. M. V. M. Soares and P. Calow, Eds.), SETAC Special Publications Series, pp. 37–55. Lewis, Boca Raton, FL.

Centeno, M. D. F., Brendonck, L., and Persoone, G. (1993a). Acute toxicity tests with *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca): Influence of selected environmental conditions. *Chemosphere* **27,** 2213–2224.

Centeno, M. D. F., Brendonck, L., and Persoone, G. (1993b). Influence of production, processing, and storage conditions of resting eggs of *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca) on the sensitivity of larvae to selected reference toxicants. *Bull. Environ. Contam. Toxicol.* **51,** 927–934.

Centeno, M. D. F., Persoone, G., and Goyvaerts, M. P. (1995). Cyst-based toxicity tests. IX. The potential of *Thamnocephalus platyurus* as test species in comparison with *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca). *Environ. Toxicol. Water Qual.* **10,** 275–282.

Charnley, A. K. (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In *Biological Control of Locusts and Grasshoppers* (C. J. Lomer and C. Prior, Eds.), pp. 181–190. CAB International, Wallingford, UK.

Cole, G. A. (1968). Desert limnology. In *Desert Biology* (G. W. Brown, Jr., Ed.), pp. 423–486. Academic Press, New York.

Coutteau, P., Brendonck, L., Lavens, P., and Sorgeloos, P. (1992). The use of manipulated baker's yeast as an algal substitute for the laboratory culture of Anostraca. *Hydrobiologia* **234,** 25–32.

Crisinel, A., Delaunay, L., Rossel, D., Tarradellas, J., Meyer, H., Saïah, H., Vogel, P., Delisle, C., and Blaise, C. (1994). Cyst-based ecotoxicological tests using anostracans: Comparison of two species of *Streptocephalus*. *Environ. Toxicol. Water Qual.* **9,** 317–326.

College voor de Toelating van Bestrijdingsmiddelen (CTB) (1999). *Bestrijdingsmiddelendatabank*. CTB Wageningen (Internet site).

Danfa, A., and van der Valk, H. C. H. G. (1999). Laboratory testing of *Metarhizium* spp. and *Beauvaria bassiana* on sahelian non-target arthropods. *Biocontrol Sci. Technol.* **9,** 187–198.

Everts, J. W., (Ed.) (1990). *Environmental Effects of Chemical Locust and Grasshopper Control: A Pilot Study*. Food and Agriculture Organization of the United Nations, Rome.

Everts, J. W. (1997). Ecotoxicology for risk assessment in arid zones: Some key issues. *Arch. Environ. Contam. Toxicol.* **32,** 1–10.

Food and Agriculture Organization of the United Nations (FAO) (1998). *Evaluation of Field Trial Data on the Efficacy and Selectivity of Insecticides on Locusts and Grasshoppers*. Report to FAO by the Pesticide Referee Group, Seventh Meeting, 2–6 March 1998. FAO, Rome.

Hansen, S. R., and Garton, R. R. (1982). Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. *Can. J. Fish. Aquat. Sci.* **39,** 1273–1288.

Hill, I. R. (1989). Aquatic organisms and pyrethroids. *Pestic. Sci.* **27,** 429–465.

Kersting, K., and Van Wijngaarden, R. (1992). Effects of chlorpyrifos on a microecosystem. *Environ. Toxicol. Chem.* **11,** 365–372.

Kooijman, S. A. L. M. (1981). Parametric analysis of mortality rates in bioassays. *Water Res.* **15,** 107–119.

Lahr, J. (1997). Ecotoxicology of organisms adapted to life in temporary freshwater ponds in arid and semi-arid regions. *Arch. Environ. Toxicol. Contam.* **32,** 50–57.

Lahr, J., Diallo, A. O., Gadji, B., Diouf, P. S., Bedaux, J. J. M., Badji, A., Ndour, K. B., Andreasen, J. E., and Van Straalen, N. M. (2000). Ecological effects of experimental insecticide applications on invertebrates in sahelian temporary ponds. *Environ. Toxicol. Chem.* **19,** 1278–1289.

LeBlanc, G. A. (1984). Interspecies relationships in acute toxicity of chemicals to aquatic organisms. *Environ. Toxicol. Chem.* **3,** 47–60.

LUBILOSA (1994). *Metarhizium flavoviride*, *Biological Insecticide for Experimental Use*, Technical and Safety Information Sheet. LUBILOSA Project, Niamey, Niger.

Maeda-Martínez, A. M., Obregón-Barboza, H., and Dumont, H. J. (1995). Laboratory culture of fairy shrimps using baker's yeast as basic food in a flow-through system. In *Studies on Large Branchiopod Biology and Aquaculture II* (D. Belk, H. J. Dumont, and G. Maier, Eds.), Developments in Hydrobiology No.103, pp. 141–157. Kluwer Academic, Dordrecht.

Mayer, F. L., and Ellersieck, M. R. (1986). *Manual of Acute Toxicity*: *Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals*, Resource Pub. No. 160. U.S. Dept. of the Interior, Fish and Wildlife Service, Washington, DC.

Mokry, L. E., and Hoagland, K. D. (1990). Acute toxicities of five synthetic pyrethroid insecticides to *Daphnia magna* and *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **9,** 1045–1051.

Moore, W. G. (1957). Studies on the laboratory culture of Anostraca. *Trans. Am. Microsc. Soc.* **76,** 159–173.

Organization for Economic Cooperation and Development (OECD) (1984). *OECD Guidelines for Testing of Chemicals*, Vols. 1 and 2. OECD, Paris.

Persoone, G. (1998). Development and validation of toxkit microbiotests with invertebrates, in particular crustaceans. In *Microscale Aquatic Toxicology*. *Advances*, *Techniques and Practice* (P. G. Wells, K. Lee, and C. Blaise, Eds.), pp. 437–449. CRC Lewis, Boca Raton, FL.

Persoone, G., Janssen, C., and De Coen, W. (1994). Cyst-based toxicity tests. X. Comparison of the sensitivity of the acute *Daphnia magna* test and two crustacean microbiotests for chemicals and wastes. *Chemosphere* **29,** 2701–2710.

Sam, S. T. (1979). A simple technique for continuous feeding of *Streptocephalus dichotomus* Baird (Crustacea: Anostraca). *J. Madurai Kamaraj. Univ.* **8,** 67–72.

Sanders, H. O., Finley, M. T., and Hunn J. B. (1983). *Acute Toxicity of Six Forest Insecticides to Three Aquatic Invertebrates and Four Fishes*, Technical Paper No. 110. U. S. Dept. of the Interior, Fish and Wildlife Service, Washington, DC.

SETAC (1996). Risk assessment in arid regions. *SETAC-Europe News* **7**(2), 5–6.

Steel, R. G. D., and Torrie, J. H. (1980). *Principles and Procedures of Statistics*: *A Biometrical Approach*, 2nd ed. McGraw–Hill, New York.

Tomlin, C., Ed. (1994). *The Pesticide Manual*, *Incorporating the Agrochemicals Handbook*, 10th ed. Br. Crop Protection Council, Farnham.

Van Rijn, J. P., Van Straalen, N. M., and Willems, J. (1995). *Handboek bestrijdingsmiddelen*: *Gebruik & milieu-effecten*. VU Uitgeverij, Amsterdam.

Visser, J. T., and Linders, J. (1992). *Bendiocarb*: *Milieu-fiche*, Adviesrapport No. 90/670101/007. College voor de Toelating van Bestrijdingsmiddelen (CTB), Wageningen.

Widianarko, B., and Van Straalen, N. M. (1996). Toxicokinetics-based survival analysis in bioassays using nonpersistent chemicals. *Environ. Toxicol. Chem.* **15,** 402–406.

Widianarko, B., and Van Straalen, N. M. (1997). Scientific research on environmental toxicology in South East Asia: A view of its position in the international arena. *Australasian J. Ecotoxicol.* **3,** 89–99.

Williams, W. D. (1985). Biotic adaptations in temporary lentic waters, with special reference to those in semi-arid and arid regions. *Hydrobiologia* **125,** 85–110.